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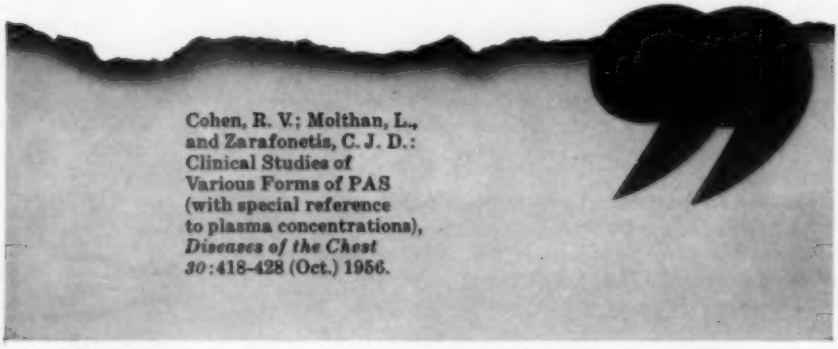

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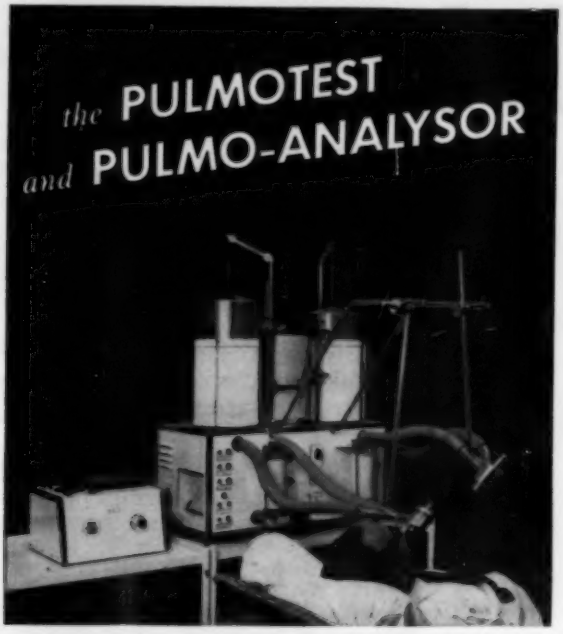

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2. Gabuzda, G. J.: Clinical and Nutritional Aspects of Lipotropic Agents, with Special Reference to Their Role in the Pathogenesis and Treatment of Fatty Cirrhosis of the Liver, Report to the Council on Foods and Nutrition, J.A.M.A. *160*:969 (Mar. 17) 1956.
3. The Committee on Dietetics of the Mayo Clinic: Mayo Clinic Diet Manual, ed. 2, Philadelphia, W. B. Saunders Company, 1954, pp. 39-41.

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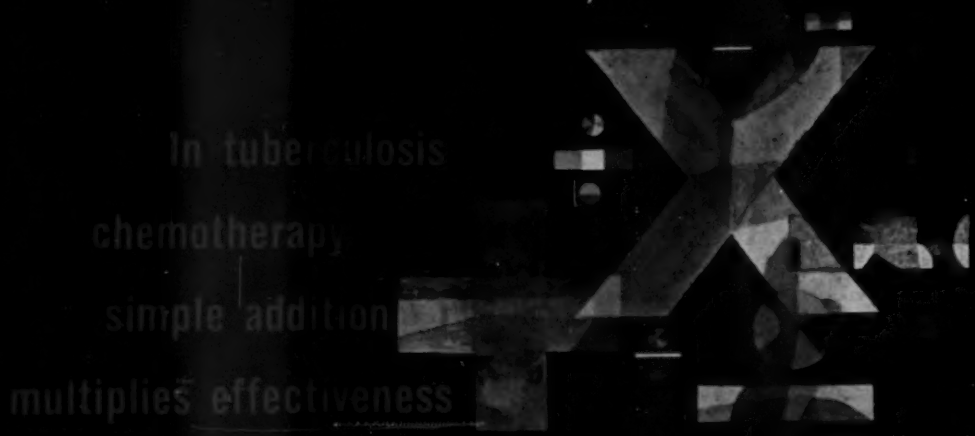

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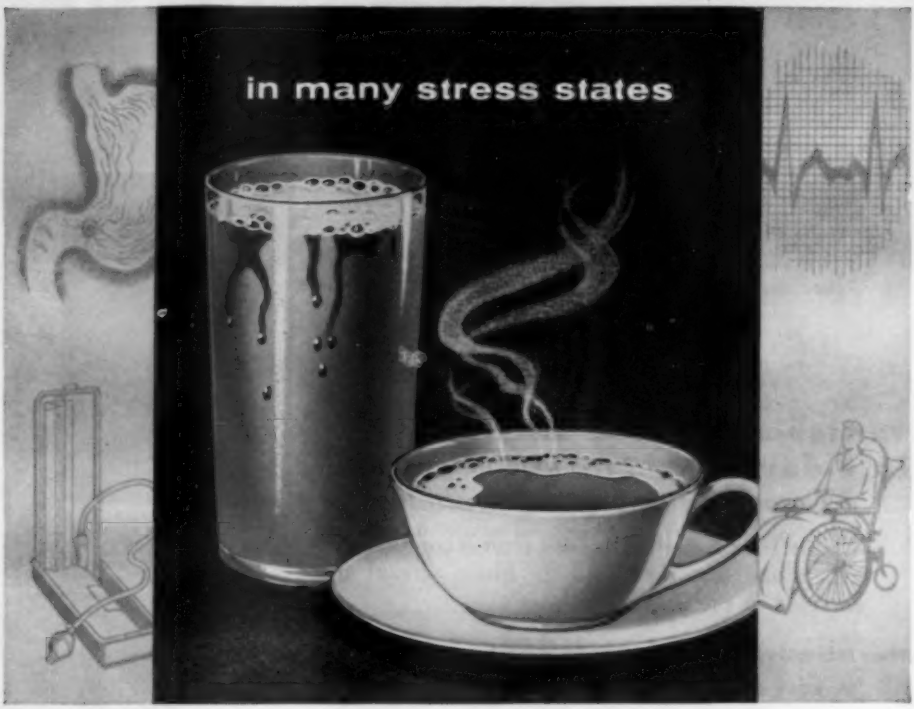

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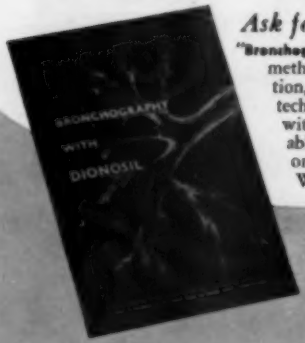

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BRONCHIAL ADENOMA[1]

A Study of 60 Patients with Resections

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Introduction

Patients who harbor a bronchial adenoma are unique in two important respects. First, they have an amazingly high rate of cure in contrast to patients with other pulmonary malignancies. Second, these patients frequently illustrate the occasional parodox noted in neoplastic disease—that the duration of symptoms has no relationship to resectability or curability.

The writers' first contact with this favorable lesion was in November, 1933, when a suppurative right lung, its bronchus obstructed by tumor, was successfully removed by the senior author (RHO). The patient, a woman of thirty-two years of age, with cough and hemoptysis, was thought to have a bronchogenic carcinoma. The final pathologic diagnosis was bronchial adenoma. Today, twenty-three years later, the patient is alive and well (1, 2). Since 1933, 59 other cases of bronchial adenoma have been seen among 1,740 patients with primary pulmonary tumors seen at the Overholt Thoracic Clinic.

Clinical Profile

Unlike bronchogenic carcinoma, this disease is more common in females and occurs at a younger age; the mean age in the present series was forty as compared with fifty for malignant tumors (tables 1 and 2). The first adenoma was reported in detail in 1882 by H. Mueller (5).[4] His patient, too, was a young woman who had suffered for eight years from a cough and fetid, blood-stained sputum.

A definite clinical pattern is followed with more regularity in bronchial adenomas than in other forms of primary lung tumor. A dry cough, unilateral wheeze, and recurrent, so-called "clean" hemoptysis are most suggestive (table

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[4] Laennec described vascular bronchial polyps in 1831.

TABLE 1

ANALYSIS OF 60 CASES OF BRONCHIAL ADENOMA TREATED SURGICALLY

Case Number	Initials	Sex	Age	Duration of Symptoms	Surgery*	Date	Pathology†	Years of Follow-Up	Remarks
				years					
1	AT	F	32	4-5/12	PN	11-33		23	Erroneously reported earlier as a cure of a lung cancer
2	EH	F	48	1	PN	5-34		22	
3	TD	F	47	5/12	PN	1-37		(15)‡	Died at height of disease, 1952
4	MC	F	37	8	PN	3-38		18	
5	SH	M	34	1	PN	5-38		18	Also reported by Holley (3)
6	MW	F	18	1-7/12	PN	1-39	Multicentric	17	
7	IS	M	48	4	PN	11-40		16	
8	NN	F	39	6	RML-RLL	7-41		15	
9	LW	F	48	18	PN	1-42		13	
10	JA	M	19	2	PN	10-42		14	
11	TC	F	15	7/12	RLL	10-43		13	
12	HS	M	51	6	PN	10-43		(2)	Died of metastases, July, 1945
13	WL	M	44	3/12	LLL	10-44		12	
14	MPut.	F	24	5/12	LLL	2-45		11	Presented as an empyema
15	MPut.	F	36	15	PN	7-45		4	Diagnosed by bronchoscopy fifteen years before surgery
16	PB	M	41	1/12	PN	5-45		11	
17	BR	F	34	5	PN	9-45	1/24	10	
18	AK	M	55	3	PN	12-45	2/21	10	
19	EB	F	25	5/12	PN	1-46		9	
20	WB	M	36	4	PN	2-46	1/3	(9)	Died at height of disease, 1955
21	FR	M	27	8	RML-RLL	4-46		9	
22	IM	M	49	1-5/12	RML-RLL	7-46		9	
23	GC	M	32	5/12	RML-RLL	9-46		9	
24	RW	M	13	1	PN	6-47		9	
25	LM	F	40	1	LUL	10-47	1/16	9	Clicking noise with respiration for five months
26	KB	F	49	3	RML-RLL	11-47		9	

27	AC	F	40	1/12	PN	4–48		8	
28	MC	F	45	3	PN	8–48		7	
29	DH	F	50	2/12	PN	2–49		6	
30	PN	M	34	20	PN	2–49		(0)	Died post operation. Tumor size, 12 × 6 × 6 cm., after twenty years made operation hazardous
31	MM	F	26	6/12	Local excision	3–49		6	Trans. pleural bronchotomy
32	MK	M	65	0	LLL	3–49		(1)	Died of bladder carcinoma
33	HB	M	45	3/12	RUL	5–49		(1)	Died of metastases, 1950, in wound, ribs, nodes, et cetera
34	JD	M	50	1/12	PN	6–49		(3)	Died of metastases, 1952. A 4 cm. lesion in RLL
35	JG	M	71	7	RLL	10–49	1/6	(4)	Died of metastases, 1953. A 7 cm. lesion in RLL
36	AL	M	58	4	PN	1–50	1/3	(0)	Died post operation, January, 1950
37	LB	F	30	0	RLL	1–50		6	Asymptomatic—in mental hospital
38	PB	F	37	2	PN	2–50	1/4	5	LMD recommended bronchoscopic control; lymph node involved at operation
39	GW	F	48	17		3–50		5	Bone and cartilage in specimen
40	MS	F	20	3	PN	4–50	Cylindromatous	5	
41	AL	F	62	4	Local excision	4–50	Multicentric	5	Two peripheral nodules thought to be metastatic carcinoma; wedge resection
42	EM	F	55	0	PN	5–50		6	Survey lesion—asymptomatic
43	RJ	F	54	45	RLL	6–50		(5)	Died of cirrhosis. Long history (forty-five years), complete relief postoperatively (1955)
44	CS	M	51	3/12	RUL	8–50		5	
45	LD	F	38	12	RLL-RML	4–51	Multicentric	4	Associated with hamartomas
46	MK	F	36	5	PN	7–51		5	
47	LB	F	40	10	PN	7–51		4	
48	AY	F	65	1	PN	2–52		4	
49	EK	M	13	5/12	RLL	3–52		4	

TABLE 1—CONTINUED

Case Number	Initials	Sex	Age	Duration of Symptoms	Surgery*	Date	Pathology†	Years of Follow-Up	Remarks
				years					
50	AA	M	31	1	PN	10–52	Cylindromatous	3	
51	TC	M	45	1-5/12	RLL	10–52		4	Operated upon for a "cyst"
52	NS	F	56	1	Local excision	2–53		3	Bronchotomy and repair with tantalum mesh
53	VF	M	31	1		8–53		(0)	Died post operation. Multicentric lesion
54	GP	M	62	1	RUL-RML	3–54		(1)	Died, 1955, of hepatic metastases
55	AF	F	39	14	PN	7–54		2	
56	FF	F	54	1-5/12	RLL	11–54		2	
57	LE	F	45	2	RML-RLL	4–55		0	
58	AS	F	43	1-5/12	RML-RLL	10–55		0	
59	NW	M	56	5/12	RLL	12–55	1/20	0	
60	FH	M	47	1	Lingula	2–56		0	

* PN: Pneumonectomy; RUL: Right upper lobectomy; RLL: Right lower lobectomy; RML: Right middle lobectomy; LLL: Left lower lobectomy; LUL: Left upper lobectomy.

† Figures in this column refer to number of nodes positive/total nodes removed.

‡ ( ): Dead.

TABLE 2

AGE AND SEX INCIDENCE OF BRONCHIAL ADENOMA

	Total Number of Cases	Males		Females		Average Age in Series
		Number	Average Age	Number	Average Age	
			years		*years*	
Moersch and McDonald (4) (Mayo Clinic, 1950)	86*	45	42	41	38	40
Present series (Overholt Thoracic Clinic)	60†	27	42	33	38	40

* Fifty-four operative cases; 5 treated by irradiation alone; 27 treated by endoscopy.
† All resected at thoracotomy.

TABLE 3

THE INITIAL SYMPTOM IN 60 CASES OF BRONCHIAL ADENOMA

Cough	22
Hemoptysis	10
Chills and fever	7
Wheeze	6
"Pneumonia"	4
Fatigue	3
"Pleurisy"	2
Colds	1
Weakness	1
Vomiting	1
Asymptomatic	3
Total	60

3). The late symptoms have been well described by Foster-Carter (6) as products, not so much of the tumor itself (as the early symptoms are), but rather of bronchial obstruction with secondary pulmonary infection. The cough becomes productive, and there may be chills and fever, pleuritic pain, dyspnea, and eventually weight loss (table 4). The picture may be similar to the findings in bronchogenic carcinoma and is distinguishable from the latter only by chronicity. The average duration of symptoms in the present and other series of adenoma was well over three years (6, 7). In fact, in some of the present cases, the symptoms existed for as long as forty-five, twenty, eighteen, seventeen, fifteen, twelve, and ten years (table 1). Curiously, all of these patients with long histories were apparently cured by resection. However, 2 patients, one with a six- and the other with a seven-year history, died two and four years after surgery. They had generalized metastases, closely resembling the original bronchial adenoma in microscopic structure. A long history does not necessarily imply benignity.

Besides having an unusually long symptomatic period, this peculiar tumor is characterized by bizarre phenomena. One patient (table 1, *Case 25*) was aware of a "clicking" sound in her chest which had been present intermittently for five months and which was synchronous with respiration. Chevalier Jackson's (8) first patient, who had an endoscopic resection in 1915, had similar symptoms

TABLE 4

RELATIVE FREQUENCY OF VARIOUS SYMPTOMS IN BRONCHIAL ADENOMA*

Symptom	Number of Cases
Cough	46
Hemoptysis	30
Recurrent hemoptysis	18
Chills and fever	28
Expectoration	26
Wheeze	12
Pain	12
Dyspnea	11
Weight loss	9
Fatigue	4
Weakness	2
Pneumonia	2
Colds	1
Vomiting	1
Asymptomatic	3

* Findings in this table are in close agreement with statistics from other published series (3, 4, 6).

with a flopping tumor which for five years had sometimes shut off inspiration and sometimes expiration with a ball-valve action. Recurrent hemoptysis during menstruation was present in another case and has been reported by various authors (3, 6, 9). One patient of the present series had an empyema necessitans which was drained. This was followed by a bronchopleural fistula, and it was only months later when lobectomy was done that the underlying adenoma was found. A similar case was reported by Foster-Carter (6).

It seems significant to mention that, when the patients could localize their trouble to one side or the other (22 cases), they were correct in every instance.

Asymptomatic bronchial adenoma was discovered by roentgenography in 3 instances.

No increased tendency to cancer, whether in the parents, siblings, or in other sites in the patients themselves, was noted in this series. There were 6 cancers in 92 parents whose history was known; 3 mothers had histories of 2 stomach and one breast malignancy, 3 fathers had cancers of the stomach in one, spine in one, and unknown origin in the third. The correlation with smoking, so marked in epidermoid tumors, was not observed in this group.

ROENTGENOGRAPHIC APPEARANCE

There was no uniformity in the roentgenographic findings. Four patients had completely normal chest films. In 3 others, the films were normal at first, and then a shadow appeared at a later date. Five tumors (8 per cent of this series) appeared as peripheral "coin" lesions. The predominant number of patients had roentgenographic changes suggesting atelectasis or inflammatory infiltrations (figure 1*A*) or, rarely, emphysema (figure 1*B* and *C*) due to bronchial obstruc-

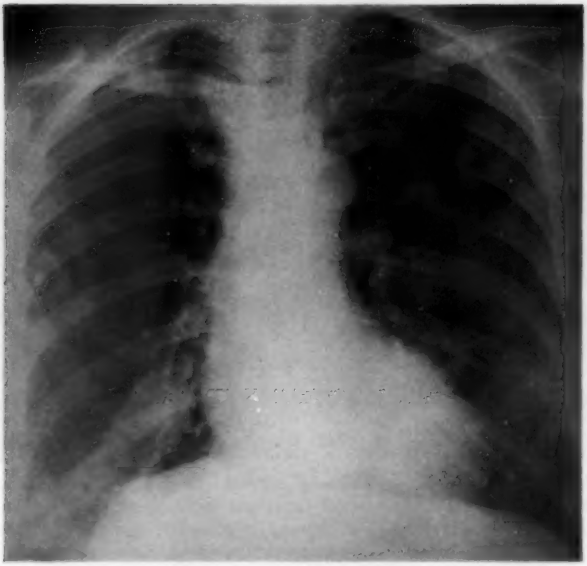

FIG. 1A. Typical roentgenogram of *Case 56*, showing a small hilar mass (the tumor) with peripheral atelectasis and pneumonitis.

tion (table 5). The roentgenographic findings varied from completely negative, as stated, or the most minimal detectable pleural effusion in the costophrenic sinus in one case, to a complete opacification of the entire hemithorax in another (figure 1*D*). Bronchography and laminagraphy were helpful, but in no case of diagnostic value.

DIAGNOSIS BY BRONCHOSCOPY

Preoperative bronchoscopy resulted in the correct diagnosis in less than half the cases (table 6). Biopsy was not done routinely when the lesion appeared to be a typical bronchial adenoma. The risk of serious hemorrhage following biopsy of this particular lesion is real, and fatalities have been reported (10).

Biopsy specimens in 7 cases were erroneously reported as showing primary bronchogenic carcinoma; and in 3 cases, as chronic inflammatory tissue. Similar rather poor results in histologic diagnosis through biopsy have been reported by Holley (3). In his series of thirty-four cases, biopsy resulted in an immediate diagnosis of bronchial adenoma in eighteen cases or slightly more than half, but in an erroneous diagnosis of carcinoma in fourteen others.

TREATMENT BY BRONCHOSCOPIC RESECTION

In two-thirds of the cases (67 per cent) invasion of the bronchial wall was present at operation. Two patients had direct extension into lymph nodes. Six more had lymph node metastases (table 7). Consequently, the writers are in

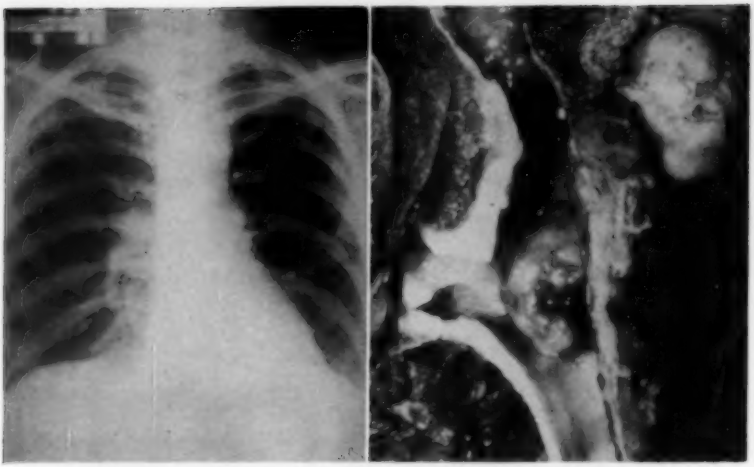

FIG. 1*B* (Left). In *Case 46*, the only roentgenographic finding was emphysema of the left lung. The patient is now well five years following pneumonectomy. *C* (Right). Photograph of left main bronchial system in *Case 46* (shown in figure 1*B*) sectioned to show intra- and extrabronchial adenoma. Note how lesion formed a check-valve to produce obstructive emphysema.

agreement with Crafoord who stated (11) that he was "of the opinion that endobronchial removal of the tumor is not to be considered as a sane procedure with the possible exception of very rare cases."

Six of the patients had been treated by bronchoscopic resection elsewhere at 16, 10, 10, 10, 3, and 2 years (average 8.2 years) before intrathoracic surgery. Each of these patients had a locally recurrent tumor at the time of operation, and one died of hepatic and generalized metastases 2 years after surgical resection, 5 years after bronchoscopic resection in another institution, and 8 years after the initial symptoms of hemoptysis (*Case 12*). Such a tragic outcome seems to have been avoided in another of the patients who underwent resection at her wish and against the advice of both her personal physician and the bronchoscopist who believed that the growth was amenable to bronchoscopic resection. Lymph node involvement was found at surgery (*Case 38*) but the patient is well 5 years later.

TREATMENT BY SURGICAL RESECTION

Usually there has been damage to the pulmonary parenchyma. The longer the history, the more extensive the permanent damage. With 3 exceptions, all patients with symptoms of more than four years' duration have required pneumonectomy. In the last decade, it has been possible to elect lobectomy more often. Two cases have been amenable to local excision by bronchotomy (table 8). The 2 patients are now well seven and four years after surgery. One of them has a tantalum gauze mesh prosthesis in the bronchial wall to replace the portion removed (figure 2). Foster-Carter (6) reported a ten-year cure following a

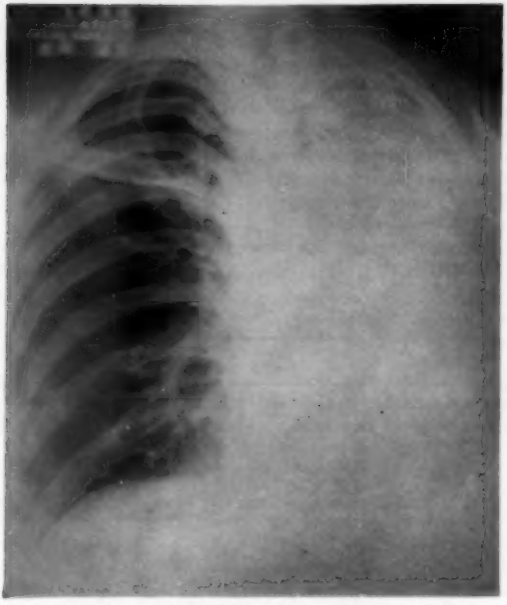

FIG. 1*D*. Total opacification of the left hemithorax in *Case 15*. There was a fifteen-year history. Because of sepsis, pleural effusion, and the poor general condition, the outcome was judged by some to be hopeless. However, the patient is now well fifteen years following pneumonectomy.

TABLE 5

ROENTGENOGRAPHIC FINDINGS IN BRONCHIAL ADENOMA

	Present Series (Overholt Thoracic Clinic)	Holley (3) (U. S. Army)	Good and Harrington (7) (Mayo Clinic)
Atelectasis	20	16	77
Inflammatory infiltration	19	9	
Tumor demonstrable as a discrete mass	8*	8	23
Roentgenograms normal	4	6	
Roentgenograms unavailable	4		
Lobar emphysema	2		
Pleural effusion	2		
Cyst	2		
	60	39	100

* Peripheral, 5; hilar, 3.

bronchotomy excision done in January, 1930. Goldman (12), Balo and associates (13), Bikfalvi and Duberz (14), and others have reported success with simple bronchotomy in selected cases. Delarue (15), however, recommends a limited pulmonary resection of the lobectomy type as the treatment of choice unless

TABLE 6

DIAGNOSIS OF BRONCHIAL ADENOMA BY PREOPERATIVE BRONCHOSCOPY AND BIOPSY

Total correct diagnosis: Bronchial adenoma	25
Immediate diagnosis bronchial adenoma (no biopsy*)	7
Immediate diagnosis bronchial adenoma (confirmed by biopsy)	12
Bronchial adenoma resected previously endoscopically	6
Total erroneous diagnosis: Malignant	11
Diagnosed malignant tumor (no biopsy)	4
Diagnosed malignant tumor on biopsy	7
Total erroneous diagnosis: Negative for tumor	24
Bronchoscopy negative	16
Bronchiectasis only	2
No bronchoscopy	6
Total	60

* In these cases the adenoma was believed to be typical so there was no question of the diagnosis. Biopsy was believed unwise in view of the vascularity of the tumor.

TABLE 7

FREQUENCY OF LOCAL INFILTRATION AND DISTANT METASTASIS IN BRONCHIAL ADENOMA

Carcinoid tumors	58	
Direct infiltration of bronchial wall	40	(67%)
Direct extension to lymph nodes	2	(3%)
"Jump" lymph node metastases	6	(10%)
Distant metastases liver, bone, et cetera	5	(9%)
Cylindromatous tumors	2	
Infiltration wall	2	
Metastases	0	

TABLE 8

PROCEDURES DONE IN 60 CONSECUTIVE CASES OF BRONCHIAL ADENOMA TREATED BY THORACOTOMY

Pneumonectomy			31
Lobectomy*	LUL—2, LLL—3	5	
	RUL—2, RML—1, RLL—7	10	15
Double lobectomy	RML—9, RLL, RUL, RML	1	10
Lingulectomy			1
Bronchotomy and plastic repair			1
Simple bronchotomy			1
Local excision by wedge resection†			1
Total			60

* See table 1 for explanation of abbreviations.

† Two wedges of two separate tumors.

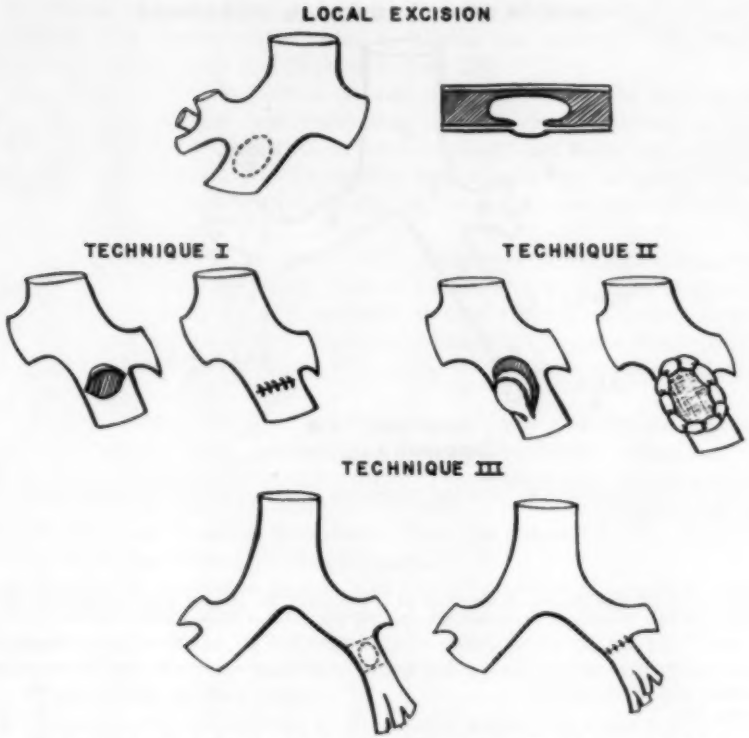

FIG. 2. Local excision in bronchial adenoma: *Technique I*—Simple removal of the endobronchial tumor and primary closure of the bronchus; *Technique II*—Replacement of the bronchial defect by fascial-wrapped sheet of tantalum mesh; *Technique III*—Excision of segment of bronchus containing the tumor with end-to-end anastomosis.

the presence of mediastinal lymph node enlargement or involvement of a main bronchus necessitates pneumonectomy. It is obvious that the extent of the resection depends not only upon the size and location of the tumor, but also upon the condition of the lung and effects of inflammatory change distal to the obstruction.

PATHOLOGIC FINDINGS

Distribution: In both this series and others the predominant number of bronchial adenomas were (*a*) central in location, (*b*) on the right side, and (*c*) in the lower lobes (figure 3 and tables 9*A* and *B*). In only 5 patients were tumors visible on roentgenography (table 7) as a discrete peripheral mass (16). The average age of these 5 patients was fifty-eight, compared with the average of forty for the series. This age difference in peripheral tumors previously was pointed out by Felton, Liebow, and Lindskog (17). Two of these 5 tumors had produced symptoms, although none was visible bronchoscopically.

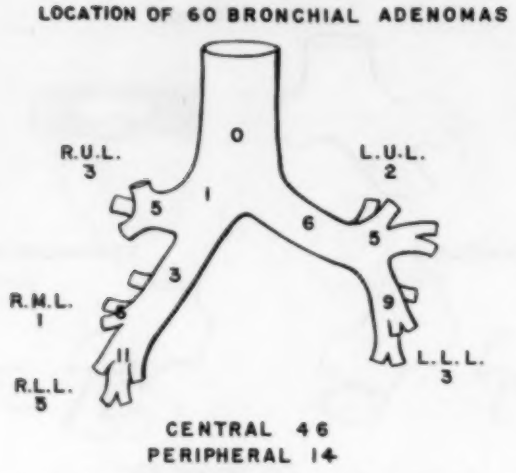

FIG. 3. Distribution of 60 bronchial adenomas. (For explanation of abbreviations, see footnote to table 1.)

TABLE 9*A*

LOCATION OF BRONCHIAL ADENOMAS BY PROXIMITY TO HILUM AND BY LATERALITY

	Right	Left	Total
Moersch and McDonald (4)	47	38	85
Holley (3)	20	19	39
	67	57	124
Present series			
Central	26	20	
Peripheral	9	5	60
	102 (55%)	82 (45%)	184

TABLE 9*B*

LOCATION BY LOBAR DISTRIBUTION

	RUL*	RML*	RLL*	RI and MB†	LUL*	LLL*	LMB‡	Totals
Moersch and McDonald (4)	7	3	19	15	9	15	14	82
Foster-Carter (6)	1	4	21	18	4	8	14	70
Present series	8	7	16	4	7	12	6	60
Total number of cases	16	14	56	37	20	35	34	212
	7%	7%	26%	17%	9%	17%	16%	100%

* See table 1 for explanation of abbreviation.

† RI and MB = Right intermediate and right main bronchus.

‡ LMB = Left main bronchus.

Production of mucus: The production of mucus in bronchial adenomas has previously been studied and reported by Gilman and associates (18), Foster-Carter (6), Mallory (19), and Engebreth-Holm (20).

Theories of origin: The theories of origin concerning bronchial adenoma fall into two groups. A source from embryologic malformation is supported by those who think of adenomas as vestigial pulmonary lobes (21) or persistent embryonal bronchial buds (22–24). Possible sources from special cellular origin include oncocytes (25), duct epithelium (19, 26), and bronchial node epithelium (4, 17, 28, and others).

Exfoliative cytology: All 10 cases in which preoperative bronchial washings were taken for cell study by the Papanicolaou technique were negative for tumor cells. This is what might be expected inasmuch as these tumors are usually covered by normal bronchial epithelium.[5] Engebreth-Holm (20) reported tumor cells in the sputum in one case in twelve. This as the only microscopically malignant-appearing adenoma in his series.

Metastases: Although in 8 of the present cases lymph node involvement was found at the time of operation, only 6 of these represented true metastases. The other 2 were direct extensions of the tumor. Two of the 6 patients subsequently died of distant metastases. Three other patients in whom no lymph nodes were positive also died of distant metastases. Thus, the metastatic potential in the present series was 15 per cent (9 of 60 cases).

Crafoord (11) reported a patient with bronchial adenoma treated by pneumonectomy in whom, after a ten-year period, the tumor recurred in the bronchial stump and mediastinum. It must be kept in mind, therefore, that a follow-up period of at least ten years may be required before cure can be considered absolute in this slowly growing tumor.

It is encouraging to note that, in the present series, 2 patients treated more than ten years ago, who had true lymph node metastases at the time, are still living.

Usually, in the cases with metastases, one can find areas under a microscope in the tumor which are more bizarre and in which the cells are anaplastic. When a bronchial adenoma develops metastases, some surgeons regard it as a carcinoma arising in a bronchial adenoma. The present writers have no quarrel with this viewpoint. However, if, as in some fatal cases included in this series, there is (*1*) a long history of symptoms, (*2*) the tumor appeared perfectly typical of a bronchial adenoma bronchoscopically at some time in the past, and (*3*) areas suggesting bronchial adenoma can be found under the microscope (29, 30), it seems proper to discuss and include the tumor with the other "bronchial adenomas."[6]

[5] It is interesting to note that as early as 1915, in Chevalier Jackson's first case report, mentioned previously (8), of a bronchial adenoma removed endoscopically, the diagnosis was suspected beforehand on the basis of examination of exfoliated cells.

[6] There have been two cases excluded from this series in which the pathologist stated that the tumor appeared to be a carcinoma, but that it might be of bronchial adenoma origin. Both of these patients had a short history. One has cerebral metastases five years after surgery, and the other is well two years after surgery.

FIG. 4. Bronchial adenoma from patient with twenty years of symptoms. The photograph shows the great extrabronchial extension of tumor to a mass of 12 by 6 by 6 cm.

Physical characteristics: Without question, some of these tumors, like a mole on the skin, stay the same size for a lifetime. One patient in the present series had had symptoms for forty-five years before surgery and, in others, the tumor had been seen by bronchoscopy up to fifteen years prior to surgery.

As suggested by Anderson (31), whenever the greatest diameter of the tumor exceeded 5 cm. (figure 4), the duration of symptoms was more than five years (table 4). On the other hand there were 2 instances in the present series in which symptoms had been present for less than two years, yet the tumors were large (more than 5 cm.). Both patients subsequently succumbed to metastatic disease.

Although bronchial adenomas are described as being typically of a pink or red color, a few were gray, tan, or white. Both cylindromas had the typical (for cylindromas) yellow, cheesy appearance.

Multicentric lesions: Four patients had multiple lesions.[7] One such was a patient (*Case 41*) who suffered from cough, wheeze, and expectoration for four years. Roentgenograms revealed a "coin" lesion in the left lower lobe. At operation, two peripheral nodules were found and removed by wedge resection. Upon frozen section, the diagnosis was metastatic carcinoma. Permanent sections caused the diagnosis to be changed to multicentric bronchial adenoma.

[7] Two other such patients with multicentric adenomas have been previously reported by Felton, Liebow, and Lindskog (17).

TABLE 10

RELATIVE FREQUENCY OF BRONCHIAL ADENOMA

	Carcinoid	Cylindromatous
Frequency		
Holley (3)	34	5
Moersch and McDonald (4)	86	9
Present series	58	2

Characteristics	
Carcinoid	Cylindromatous
Common	Rare
Central	Central even tracheal
Reddish, lobulated	Yellow, "cheesy"
Younger age group	Older age group
60 per cent infiltrate, bronchial wall	100 per cent infiltrate, bronchial wall
10 per cent malignancy mortality	Up to 50 per cent mortality (Moersch and McDonald (4))

The finding of multiple foci of epithelium and muscle in another case in which multiple bronchial adenomas were present lends credence to Womach and Graham's concept (22) that the origin of bronchial adenoma is similar to that of Albrech's hamartoma. They state also that bronchial adenoma is frequently associated with other anomalies of fissuring and bronchovascular distribution. The present writers have observed no such correlation and, in fact, have noted only one anomaly among 60 cases.

Cylindromatous type: The cylindromatous type of bronchial adenoma is much rarer than the carcinoid variety (table 10). In each of the 2 cases in the present series, the tumor was pale, yellow, firm, lobulated, and extended diffusely in the bronchial wall. The present writers agree with Rabin (32) that this tumor should be considered separately from the ordinary carcinoid (table 10 and figure 5).

RESULTS OF SURGERY IN BRONCHIAL ADENOMA

Forty-eight of the 60 patients are living and well. Of the 12 dead, 5 died of metastases at one, one, two, three, and four years postoperatively. Four died of intercurrent disease at one, five, eleven, and fifteen years postoperatively. Three were postoperative deaths (table 11).

The first 20 patients have passed the critical ten-year mark (35). There was no operative mortality in this group on which resection was done in the period 1933 to 1945, and only one succumbed to the tumor, although 3 others had lymph node involvement. Although 2 patients have died of heart disease after nine and fifteen years, the fact remains that for these 20 patients, a 95 per cent rate of cure has been possible with surgery. As mentioned, and oddly enough, there was no operative mortality (0 per cent) in this group of resections done

FIG. 5.A (Left). Photomicrograph from *Case 14*, carcinoid type, in 24-year-old female now well eleven years following surgery. *B* (Center). Photomicrograph of cylindromatous type of tumor (*Case 50*). *C* (Right). Photomicrograph from *Case 25* in which one lymph node was positive for tumor at exploration. The adenoma cells can be seen invading cartilage. The patient is well nine years after left upper lobectomy.

TABLE 11

SUMMARY OF FOLLOW-UP DATA*

Total dead	12
Dead of metastases (at 1, 2, 2, 3, and 4 years post operation)	5
Died postoperatively (at 24, 24, and 48 hours)	3
Died of intercurrent disease (at 1, 5, 11, and 15 years)	4
Total living (all apparently cured)	48
Less than 5 years living and well	15
5–10 years post operation	17
11–20 years post operation (inclusive)	13
More than 20 years post operation	2
	60

* All surviving patients have been followed to January, 1956.

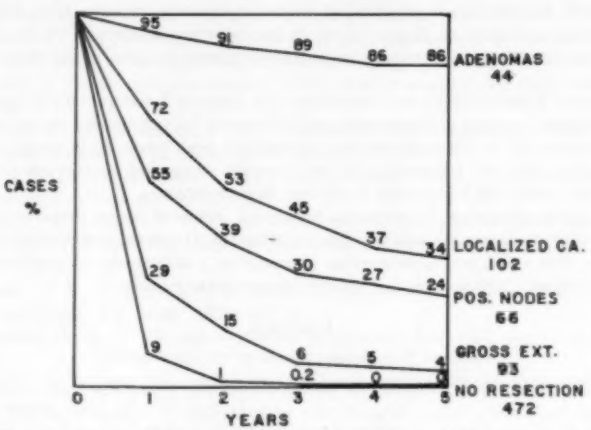

FIG. 6. The comparison of five-year survival rates of bronchial adenoma with other lung tumors.

twenty-two to ten years ago. The over-all operative mortality rate for the entire series was 5 per cent.

A comparison of survival rates for bronchial adenoma and other forms of primary malignant lesions of the lung is shown in figure 6. This experience clearly indicates that bronchial adenoma is the most favorable of any primary lung tumor which carries a malignant potential (33–37).

SUMMARY

Bronchial adenoma produces a characteristic clinical picture. Its usual prolonged course bears no relationship to curability. A definite malignant potential is present and is evidenced in approximately 15 per cent of the cases by lymph node and distant metastases. Occasional patients have multicentric lesions (4 in the present series).

Bronchoscopic resection is not a reliable method of treatment. Cure requires transthoracic excision of the tumor and lymph node removal. The extent of lung resected depends largely upon the extent of irreversible changes in the pulmonary tissue. Early intervention will conserve lung and, in some cases, will permit local excision by bronchotomy.

Treated surgically, bronchial adenoma has the best prognosis of any lung tumor with a malignant potential. Of the first 20 consecutive patients operated upon more than ten years ago, there were no operative deaths and only one patient later succumbed to the tumor. The remaining 95 per cent appear to be cured.

Sumario

Adenoma Bronquial: Estudio de 60 Casos Resecados

El adenoma bronquial produce un típico cuadro clínico. Su habitual evolución prolongada no guarda relación con la curabilidad. Exite un potancial maligno bien definido que se traduce aproximadamente en 15 por ciento de los casos por metástasis en los ganglios linfáticos y remotas. De vez en cuando los enfermos tienen lesiones multicéntricas (4 en la serie actual).

La resección broncoscópica no constituye un método fidedigno de tratamiento. La curación requiere la excisión transtorácica del tumor y la extirpación de los ganglios linfáticos. La extensión de pulmón resecado depende en gran parte de la de las alteraciones irreversibles en el tejido pulmonar. La intervención temprana conservará pulmón y, en algunos casos, permitirá la excisión total con la broncotomía.

Tratado quirúrgicamente, el adenoma bronquial ofrece el mejor pronóstico de ningún tumor pulmonar de potencial maligno. En los primeros 20 enfermos consecutivos operados hace más de diez años, no hubo muertes operatorias y solamente un paciente sucumbió después del tumor. El 95 por ciento restante parece estar curado.

Resume

Adénome bronchique: Etude de 60 cas d'exérèse

L'adénome bronchique produit un tableau clinique caractéristique. Son évolution généralement prolongée est sans rapport avec sa curabilité. Il comporte un réel potentiel de malignité, qui est démontré par la présence d'adénites et de métastases à distance dans approximativement 15% des cas. Occasionnellement, 4 dans la série actuelle, les malades sont porteurs de lésions à foyers multiples.

La résection bronchoscopique n'est pas un procédé thérapeutique sûr. La cure doit consister en excision transthoracique de la tumeur avec curage ganglionnaire. L'étendue de la résection dépend largement de l'étendue du tissu pulmonaire affecté de lésions irréversibles. L'intervention précoce préserve le poumon et, dans certains cas, permettra l'excision locale par bronchotomie.

Le pronostic de l'adénome bronchique traité chirurgicalement est un des meilleurs parmi les néoplasies pulmonaires ayant un potentiel de malignité. Sur les 20 premiers cas consécutifs opérés il y a plus de dix ans, il n'y a pas eu de décès opératoire et un seul des malades est mort ultérieurement, ce décès est imputable à la tumeur. Parmi les autres cas restants, 95% paraissent guéris.

REFERENCES

(1) Overholt, R. H.: The total removal of the right lung for carcinoma, J. Thoracic Surg., 1934, *4*, 196 (*Case 1*).

(2) Overholt, R. H.: Apparent cure of primary carcinoma of the lung by pneumonectomy: Case report of a five-year follow-up study, J.A.M.A., 1939, *113*, 673 (*Case 1*).

(3) Holley, S. W.: Bronchial adenomas, Mil. Surgeon, 1946, *99*, 528.
(4) Moersch, H. L., and McDonald, J. R.: Bronchial adenoma, J.A.M.A., 1950, *142*, 297.
(5) Mueller, H.: Zur Entstehungsgeschichte der Bronchialweiterungen, Inaug. Dissert Ermsleben, H. A. Busch, 1882.
(6) Foster-Carter, A. F.: Bronchial adenoma, Quart. J. Med., 1941, *10*, 139.
(7) Good, L. A., and Harrington, S. W.: Asymptomatic bronchial adenoma, Proc. Staff Meet., Mayo Clin., 1953, *23*, 577.
(8) Jackson, Ch.: Endothelioma of the right bronchus removed by peroral bronchoscopy, Am. J. Med. Sc., 1917, *153*, 371.
(9) Jackson, C. L., and Konzelmann, F. W.: Bronchoscopy aspects of bronchial tumors with special reference to bronchial adenoma, J. Thoracic Surg., 1937, *6*, 312.
(10) Waterman, D. H.: Discussion of papers of Drs. Goldman, Rabin, and Newhof on bronchial adenomas, J. Thoracic Surg., 1949, *18*, 158.
(11) Crafoord, C.: Discussion of papers of Drs. Goldman, Rabin, and Newhof on bronchial adenoma, J. Thoracic Surg., 1949, *18*, 159.
(12) Goldman, A.: Transpleural bronchotomy, J. Thoracic Surg., 1952, *23*, 237.
(13) Balo, J., Kassay, D., and Bikfalvi, A.: Les Adenomas Bronchiques et Leur importance, Bull. Assoc. franç. p. l'étude du Cancer, 1955, *42*, 76.
(14) Bikfalvi, A., and Duberz, S. S.: Resection and anastomosis of the bronchus carried out in a case of benign tumor, J. Thoracic Surg., 1955, *29*, 888.
(15) Delarue, N. C.: Bronchial adenoma: Analysis of 26 cases, J. Thoracic Surg., 1951, *21*, 535.
(16) Maier, H. C., and Fischer, W. W.: Adenoma arising from small bronchi not visible bronchoscopically, J. Thoracic Surg., 1947, *16*, 392.
(17) Felton, W. L., II, Liebow, A. A., and Lindskog, G. E.: Peripheral and multiple adenomas, Cancer, 1953, *6*, 555.
(18) Gilman, R. A., Scarpelli, D. G., and Klassen, K. D.: Mucous gland adenoma of the bronchus: A case with histo-chemical study of secretion, Am. J. Clin. Path., 1956, *26*, 151.
(19) Mallory, T. B.: Case records of the Massachusetts General Hospital, Case 352272, New England J. Med., 1949, *241*, 30.
(20) Engebreth-Holm, J.: Benign bronchial adenomas, Acta chir. Scandinav., 1944, *90*, 383.
(21) Hochberg, L. A., and Schachter, B.: Benign tumors of the bronchus and lung, Am. J. Surg., 1955, *89*, 425.
(22) Womach, N. A., and Graham, E. A., Mixed tumor of the lung, so-called bronchial or pulmonary adenomas, Arch. Path., 1938, *26*, 165.
(23) Brock, R. C.: Pulmonary pathology, diagnosis, new growths, and treatment, Lancet, 1938, *2*, 1041.
(24) Price, T. C.: Adenoma of the bronchus, in *Malignant Disease and Its Treatment by Radium*, by S. Cade, Williams & Wilkins Co., Baltimore, Md., 1940, p. 698.
(25) Stout, A. P.: Cellular origin of bronchial adenoma, Arch. Path., 1943, *35*, 803.
(26) Wessler, H., and Rabin, C.: Benign tumors of the bronchus, Am. J. Med. Sc., 1932, *183*, 164.
(27) Moersch, H. J., Tinney, W. S., and McDonald, J. R.: Adenoma of the bronchus, Surg., Gynec. & Obst., 1945, *81*, 551.
(28) Kramer, R.: Adenoma of the bronchus, Ann. Otol., Rhin. & Laryng., 1930, *39*, 689.
(29) Asada, A., and Nakamura, K.: Bronchogenic adenocarcinoma developing in a central adenoma: Report of a case, Bull. Osaka Med. School, 1955, *2*, 14.
(30) Umiker, W., and Storey, C. F.: Adenocarcinoma developing in peripheral bronchial adenoma case, J. Thoracic Surg., 1952, *24*, 420.
(31) Anderson, W. M.: Bronchial adenoma with metastasis to the liver, J. Thoracic Surg., 1942-3, *12*, 351.
(32) Rabin, C. B.: Discussion, J. Thoracic Surg., 1949, *18*, 162.

(33) Adams, W. E., Steiner, P. E., and Block, R. G.: Malignant adenoma of the lung: Carcinoma-like tumors with long clinical course, Surgery, 1942, *11*, 503.

(34) Goldman, A.: The malignant nature of bronchial adenoma, J. Thoracic Surg., 1947, *18*, 137.

(35) McBurney, R. P., Kirklin, J. W., and Woolner, L. B.: Metastasizing adenomas, Surg., Gynec. & Obst., 1953, *96*, 482.

(36) Overholt, R. H., and Schmidt, I. C.: Cure rate in lung cancer: Review of 27 adenoma cases, New York State J. Med., 1949, *49*, 910.

(37) Chauvet, M., and Lasserre, R.: A Propos Des Adenomes Bronchiques de Jackson, Schweiz. med. Wchnschr., 1955, *85*, 42.

PULMONARY TUBERCULOSIS DEVELOPING IN PERSONS OBSERVED FROM CHILDHOOD[1,2]

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Introduction

This study is based on the examination of 1,525 children, aged thirteen or less, who attended the Ealing Chest Clinic for the first time during the years 1942–1945. Some had newly diagnosed cases of tuberculosis. Others were referred by private doctors or public health agencies, usually on account of symptoms suggestive of tuberculosis. The greatest number of children were asymptomatic and came because of contact with known, often newly discovered, tuberculosis, usually in a relative.

Routine examination included a tuberculin patch test followed, if negative, by a Mantoux test of 100 mg. of OT or its equivalent in PPD. All positive reactors and all children with symptoms had chest roentgenograms. Contact children who were initially Mantoux negative were re-tested (patch) at three-month intervals so long as contact was maintained or until conversion occurred. (This was in the days before BCG was available in Great Britain.) Children who were found to have, or who later developed, manifest tuberculosis were classified and treated accordingly.

There were some cases in which, for one reason or another, tuberculin testing was incomplete and some in which not even a patch test was done. This occurred more in older children, who had roentgenograms in any case, and those, for example, with calcified lesions or otherwise presumed to have already been infected. However, the figures were sufficient to give a fair picture of tuberculous infection and disease in a child population, as seen from a chest clinic (1).

Five years later (2) a follow-up was conducted through the records at the clinic. It was found that, in addition to the 7 children who had died as a direct outcome of their primary infection, all within a few months of tuberculin conversion, death had occurred in one of 3 children discovered at the outset to have advanced bilateral pulmonary tuberculosis. (The other 2 died later.) There were no further deaths from tuberculosis, but 3 children died from other causes in the earlier observation period, and one died subsequently.

In 97 patients in whom there had been a roentgenographically recognizable lesion within the thorax, including 28 with segmental or lobar collapse, none had residual ill health except one patient with asthma and one with symptomatic bronchiectasis. The primary lesions had become calcified, fibrotic, or invisible. In 3 cases a local bronchiectasis was either suspected or proved, but was accompanied by no significant symptoms.

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[2] Presented at a meeting of the British Tuberculosis Association in Dublin, Eire, on May 4, 1956.

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This experience seemed to show that, at least in the population concerned, primary tuberculous infection, whether or not attended by manifest lesions, was in general benign. Apart from an initial mortality from meningitis, which occurred before the introduction of streptomycin, all patients made a good recovery during childhood without subsequent ill effects. It remains to be seen how their later life may be affected.

Plan of Study

Since the publications quoted above, the records of the 1,511 children surviving have been studied each year, with the object now of discovering any who have developed chronic pulmonary tuberculosis. Up to June, 1956, there have been 31 such cases. Only 5 of these cases of chronic pulmonary tuberculosis were diagnosed as a result of the patients' symptoms. Seventeen were detected in routine follow-up roentgenograms and 9 by mass roentgenography, 4 of the latter through call-up for national service. Other cases may have occurred without being detected, for many of the original children have now ceased to attend the clinic routinely. There is now a great deal of routine roentgenography of the chest, in terms of employment or for national service. Moreover, the parents are alert to the possibility of tuberculous infection, especially when a chest clinic has been attended in the past. Consequently, any lesions in this series are likely to be undetected only if spontaneously arrested at an early stage. On the other hand, it is improbable that any case actually diagnosed has been overlooked, even in persons changing name through marriage or moving to another area, because reference back to the previous history is always recorded. In instances in which inquiries have come from other chest clinics regarding persons who had attended the Ealing Clinic in the period under review they have been investigated, and one person was found in whom pulmonary tuberculosis had developed (*Case 12*).

It cannot be claimed that this is a very satisfactory follow-up, based as it is to some degree on negative evidence. Even the tuberculin testing would not stand up to present-day standards: the patch test is obsolete and it is now known that Mantoux 1:100 is liable to give false positive reactions. These deficiencies have been taken into account in what follows, especially in the extent to which statistical comparisons can be pressed.

Observations

A schematic presentation of the 31 cases of pulmonary tuberculosis is given in table 1. There were 24 girls and 7 boys. Twenty-four came initially under observation as contacts of patients with human tuberculous infection. Only one of these had a manifest primary lesion at entry (*Case 12*), and another (*Case 23*) developed such a lesion two years later. The other 7 children originally came to the clinic with symptoms. Three had acute pleurisy with effusion and one, an active primary complex. The remaining 3 had no evidence of disease.

There were 83 children in the whole series who had manifest primary intra-

thoracic lesions and survived them; only 2 of these subsequently developed pulmonary tuberculosis. Three other cases occurred in the group of 15 children who initially had pleurisy with effusion.

Seventeen children were tuberculin positive when first examined and 2 were Mantoux negative. Of 3 others negative to patch test only, 2 became tuberculin reactors at a later time (*Cases 6* and *23*). Nine had no tuberculin test; these were all older children or children in close contact with an infectious case and judged likely to be already positive.

It will be seen that after initial examination ("entry" to this study) the continuation and closeness of observation varied a good deal. The sort of variation in the group developing tuberculosis could also be seen in the group as a whole. Those in contact with tuberculosis usually attended regularly over long periods, while children supposedly at no special risk were not followed routinely. However, it seems that having once attended a chest clinic does suggest repetition of the experience to a patient or his parents. It was found that a very high proportion of all the children, whether deliberately followed or not, did in fact continue to attend occasionally later on. This was fortunate for the present study, in that 21 of the total of 31 patients were found to have had a normal-appearing chest roentgenogram within two years of the detection of the lesion and 5 others, within three years. Thus the date of onset could be determined with fair accuracy in all but 5 cases, and of these 5 only *Case 20* (the oldest person in the series, a man of twenty-three years) showed disease apparently of some years' duration.

The incidence of discovered pulmonary tuberculosis in each year of observation is shown in table 2. The yield year by year was fairly even up to 1953, when there was a big rise. Since that year, there have been no further cases. The age at which pulmonary tuberculosis was discovered is dealt with in more detail later (*see* table 6).

Character of the Lesion

Pulmonary tuberculosis, when first detected, was minimal in extent in all except 2 cases (*Diagnostic Standards*, National Tuberculosis Association (United States)). In 14 cases it was bilateral. The earliest lesion was predominantly apical or subapical, except in *Cases 14* and *27*, described later.

Thus far there have been no deaths, but 8 patients are still under active treatment. Had the benefits of modern chemotherapy come at an earlier date, some of the lesions might have been better controlled. As it is, the disease in 10 has reached a moderately advanced stage and in one there has also been involvement of the knee joint. Cavity formation has been demonstrated in 22, and in 15 cases tubercle bacilli have been recovered from the sputum. Fourteen patients have had collapse therapy, 2 undergoing major surgery.

Chronic pulmonary tuberculosis with caseous, fibrocaseous, or finely nodular lesions, waxing and waning with the years and undergoing from time to time bronchogenic extension, was the usual pattern, but 3 cases were somewhat different and require description.

TABLE 1

THIRTY-ONE CHILDREN, FIRST SEEN IN 1942-1945, WHO LATER DEVELOPED PULMONARY TUBERCULOSIS

Tuberculin Sensitivity, Contact Status, and Roentgenographic Appearances on Entry and on Each Successive Anniversary up to the First Detection of Disease

Case Number	Sex	Age	Condition on Entry		Condition, Including Chest Film, on Each Successive Anniversary												Known Contact with Infectious Tuberculosis
			Tuberculin Test	Chest Film	1	2	3	4	5	6	7	8	9	10	11	12	
		yrs.															*years*
(1942)																	
1	M	11	Patch+*	Normal	Film normal	Film normal	Film normal	Film normal	Film normal	Film normal	Film normal	Infiltration right apex					On entry and through 3rd
2	F	7	Patch+	Normal	Film normal	Film normal	Infiltration left upper lobe; sputum +; tuberculous left knee joint										On entry and through 1st
3	M	11	No test	Normal	Film normal	Film normal	Unknown	Unknown	Unknown	Infiltration both apices; sputum+							On entry and through 3rd
4	M	10	Patch+	Normal	Film normal	Unknown	Infiltration both apices										On entry only
5	F	12	No test	Normal	Unknown	Unknown	Unknown	Unknown	Unknown	Film normal	Unknown	Unknown	Cavitary lesion right upper lobe				On entry and through 8th
6	F	8	Patch−†	Normal	Patch +, film normal	Unknown	Unknown	Unknown	Unknown	Calcification left hilum	No change on film	No change on film	Unknown	Unknown	Unknown	Infiltration right apex	On entry and through 9th
(1943)																	
7	F	13	No test	Normal	Film normal	Film normal	Film normal	Infiltration both upper lobes; sputum +									On entry and through 2nd
8	F	8	Patch+	Calcification left hilum	No change on film	No change on film	No change on film	Unknown	Unknown	Unknown	Unknown	No change on film	Infiltration both apices				On entry and through 8th
9	M	12	Mantoux+	Normal	Unknown	Unknown	Unknown	Unknown	Unknown	Infiltration both apices; sputum +							On entry and through 8th
10	F	12	No test	Normal	Film normal	Unknown	Unknown	Unknown	Film normal	Unknown	Unknown	Unknown	Infiltration left upper lobe; sputum +				None
11	F	8	Patch+	Left pleural effusion	Film normal	Film normal	Calcification left apex and hilum	No change on film	No change on film	Unknown	Unknown	No change on film	Infiltration left apex				None
12	F	6	Patch+	Hilar lymph node enlargement	Hilar calcification, left	No change on film	No change on film	No change on film	No change on film	Unknown	Unknown	Unknown	Film normal	Infiltration both apices; sputum+			None
13	F	5	Patch+	Normal	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Film normal	Cavitary lesion right upper lobe; sputum+				On entry and through 8th
14	F	12	No test	Calcified primary complex left	No change on film	No change on film	Unknown	No change on film	Cavitary lesion right upper lobe								On entry and through 4th

15	F	10	Mantoux--	Normal	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Infiltration left upper and right middle zones				None
16	F	8	Patch+	Normal	Film normal	Film normal	Film normal	Film normal	Film normal	Film normal	Infiltration right apex					On entry and through 6th
17	F	12	Mantoux+	Normal	Film normal	Film normal	Film normal	Unknown	Unknown	Unknown	Cavitary lesion left upper lobe; sputum+					On entry and through 3rd
18	M	13	No test	Normal	Unknown	Film normal	Unknown	Cavitary lesions both upper lobes; sputum+								On entry and through 3rd
19	F	4	Mantoux+	Normal	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Cavitary lesion right upper lobe		On entry only
20	M	13	Mantoux+	Normal	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Infiltration throughout both lungs; sputum+		On entry only
21	M	8	Mantoux-	Normal	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Infiltration left upper lobe; sputum+		None
(1944)																
22	F	13	Patch+	Right pleural effusion	Infiltration left upper lobe; sputum+											None
23	F	6	Patch-	No film	No film	Patch+; hilar lymph node enlargement	Film normal	Infiltration both apices								On entry and through 3rd
(1945)																
24	F	12	Patch+	Normal	Film normal	Infiltration and cavity, right upper lobe; sputum+										On entry
25	F	13	No test	Normal	Film normal	Film normal	Film normal	Infiltration both upper lobes; sputum+								On entry and through 1st
26	F	11	Patch+	Calcification left hilum	No change on film	Unknown	Infiltration left upper lobe									On entry and through 2nd
27	F	13	Patch+	Left pleural effusion	Hilar calcification, right	No change on film	No change on film	Round focus right middle zone								None
28	F	9	Patch-	No film	Unknown	Unknown	Infiltration both apices; sputum+									On entry and through 1st
29	F	10	No test	Normal	Film normal	Film normal	Film normal	Film normal	Film normal	Film normal	Infiltration left upper lobe					On entry and through 6th
30	F	8	Mantoux+	Calcification right hilum	Unknown	Unknown	Film normal	Film normal	Film normal	Film normal	Unknown	Infiltration right upper lobe				On entry and through 6th
31	F	13	No test	Normal	Film normal	Film normal	Unknown	Unknown	Unknown	Unknown	Film normal	Cavitary lesion right upper lobe				On entry and through 3rd

* + = Positive.
† − = Negative.

TABLE 2

AGE AND YEAR OF DIAGNOSIS

Age	Years									Total
	1945	1946	1947	1948	1949	1950	1951	1952	1953	
years										
8–14	3	1	—	3	—	—	—	1	1	9
15–19	—	1	1	2	3	3	1	2	6	19
20–23	—	—	—	—	—	—	1	—	2	3
Total.....	3	2	1	5	3	3	2	3	9	31

Case 11, a girl aged eight, had a left-sided pleural effusion in 1943. Three years later, a calcified nodule appeared in the left lung apex, with calcification also at the left hilum, presumably the primary complex showing up for the first time with increasing roentgenographic density. No further change took place until 1952, when a round solid focus, 3 cm. in diameter, appeared suddenly in the left apex, adjacent to the calcified nodule.

Case 27, a girl aged thirteen, had a right pleural effusion in 1945. As the fluid regressed, a shadow, 3 cm. in diameter, came into view in the right middle zone, interpreted as a cavitated primary focus. It became fainter and disappeared, repeated roentgenograms during 1946–1948 being normal. In 1949, a round solid focus of the same size suddenly appeared in the exact site of the previous shadow. This is still present six years later, without softening or spread.

In these 2 cases, the subsequent pulmonary lesions seen to have arisen as a direct outcome of the primary lesion, with different periods of latency. In neither case have they yet given place to progressive disease or bronchogenic dissemination, yet the picture suggests a link between primary infection and chronic pulmonary tuberculosis. One further case may fall into this group.

Case 14, a girl aged twelve, was examined in 1943 because of hemoptysis. No tuberculin test was done, but the chest film was normal then and in the following year, and again in 1948, when symptoms recurred. In 1952, on examination as part of a mass roentgenographic survey, she was found to have a small lesion, 2 cm. in diameter, running up from the left hilum. Tomography showed the round component to contain a cavity, and laryngeal swabs grew tubercle bacilli. This was highly suggestive of a reactivated primary lesion, previously invisible on roentgenography.

Age and Sex

The overwhelming preponderance of girls in those developing pulmonary tuberculosis is not due to any sex bias at the onset. The proportion of boys to girls was approximately equal in the original group of children, and was equal in the subgroups arranged by age, by contacts and noncontacts, by tuberculin reaction, and by the incidence of overt primary and extrapulmonary tuberculous lesions. The statistical analyses that demonstrate this point have been omitted in order to simplify the other tables.

The distribution by age at the date of entry of 1,511 survivors is shown in table 3, together with the number in each group that later developed pulmonary

tuberculosis. There was an average entry for each year of age of 85–120 children. Of 31 cases of pulmonary tuberculosis, 19 occurred in children more than ten years of age when first seen and, indeed, 13 were in children aged twelve or more.

Annual attack rates may be calculated by assuming an average observation period of twelve and one-half years, which is the mean of the period 1942–1945 through 1956. In this way, table 4 can be developed to show that the incidence of subsequent pulmonary tuberculosis rose from none in those children entering in the first year of life to 3.6 per thousand of those entering in preadolescence, i.e., before the fourteenth birthday. These values have only a statistical reality, because morbidity was not spread evenly over the years, as is shown later.

The present ages of the 1,511 survivors appear in table 5. It may be seen that most of the children have already passed puberty and more than half can be regarded as adults. The youngest children, aged less than one year on entry in 1945 (midyear), are now aged eleven, and the oldest, aged thirteen in 1942, are now twenty-seven.

TABLE 3
AGE ON ENTRY OF 1,511 SURVIVORS

Years of Age	Less than 1	1-4	5-9	10-13	Total
Total survivors	85	380	625	421	1,511
Cases of pulmonary tuberculosis	—	1	11	19	31
Percentage in each age group of total cases	—	3.2	35.5	61.3	100.0

TABLE 4
ANNUAL ATTACK RATE, CALCULATED ON A MEAN OBSERVATION OF 12.5 YEARS

Age on Entry	Total Survivors	Cases of Pulmonary Tuberculosis	Rate per cent 1942-1945 Through 1956	Rate per 1,000 per Annum
years				
Less than 1	85	—	—	—
1–4	380	1	0.3	0.24
5–9	625	11	1.7	1.36
10–13	421	19	4.5	3.60
Total	1,511	31	2.0	1.60

TABLE 5
PRESENT AGE OF 1,511 SURVIVORS

Years of Age	11-14	15-19	20-24	25-27	Total
Tuberculin test positive on entry	151	315	279	123	868
Other	75	267	241	60	643
Total	226	582	520	183	1,511

TABLE 6
AGE INCIDENCE OF PULMONARY TUBERCULOSIS

Years of Age	Number at Risk	Number of Cases	Rate per 1,000
8–13	1,511–1,446	4	0.5
14	1,380	5	3.6
15	1,285	1	0.8
16	1,187	5	4.2
17	1,079	7	6.5
18	959	4	4.2
19–23	828–382	5	2.3

The group regarded as initially tuberculin positive includes children not actually tuberculin tested, but with roentgenographic evidence of calcified foci, tantamount in this population to previous tuberculous infection (3). The remainder comprises children negative to patch and Mantoux tests, together with those in whom testing was incomplete and those who were not tested at all, but showed no calcified foci. The groups "tuberculin test positive" and "other" are therefore only approximate in estimation of tuberculin-sensitivity state.

In order to show the incidence of pulmonary tuberculosis at each year of age, table 6 has been constructed, giving the number diagnosed against the number at risk during that year, a number which decreases annually after the age of eleven. All surviving children have reached that age, but the number reaching each successive year of age thereafter becomes progressively less.

It seems clear that the ages fourteen to eighteen were by far the most dangerous, and concentration into this age group might have been more pronounced but for the delay in diagnosis in some cases already mentioned. No case occurred in a person more than twenty-three years of age. There was no substantial difference in age incidence between boys and girls.

Tuberculin Sensitivity

In considering table 5, the limited value of the definitions "tuberculin test positive" and "other" was emphasized. Of 868 children in the former group, 20 later developed pulmonary tuberculosis (2.3 per cent), compared with 11 cases in 643 children in the latter group (1.7 per cent).

Of the 31 persons who developed pulmonary tuberculosis, 17 were tuberculin positive on entry and 2 others "converted" from negative to positive within a few years (*Cases 6* and *23*).

Including the 2 who later "converted" only 5 of the 31 were initially tuberculin negative, 3 of them to patch test only, the Mantoux not being done. The remaining 9 were not tested at all; these were older children, of whom 8 were in known contact with tuberculosis and were thought almost certainly to be positive.

It appears, therefore, that the pulmonary tuberculosis that subsequently occurred was only exceptionally the result of a primary infection incurred after entry. In other words, the great majority of these patients were primarily in-

fected during childhood, chronic pulmonary tuberculosis supervening after a lapse of time. This time lapse was usually several years (*see* table 1), even eight or more, though there were instances (*Cases 22* and *24*), in which it was less than two years. In these cases, it is easier to imagine the pulmonary tuberculosis as arising directly from the primary infection than in the others. It is noteworthy that both were girls, on entry aged twelve and thirteen, respectively, and one had pleurisy with effusion of the type in which directly progressive disease can often be demonstrated (4).

Otherwise the connection between primary infection and subsequent pulmonary tuberculosis was remote in time and lacking in direct evidence. The 2 cases described earlier, in which the subsequent lesion was a solitary focus arising near, or at the site of, the primary lesion (*Cases 11* and *27*) are perhaps in a special class, as also may be *Case 14*, in which the lesion was apparently a recrudescence of the primary focus.

Contact with Tuberculosis

Of all the 741 children originally in contact with infectious tuberculosis, a permanent break in this contact, in war-time conditions and with less effective treatment than is available today, was often not achieved. The patient with tubercle bacilli in the sputum might die, or his disease become arrested, or he might be temporarily segregated in a hospital. If not effectively treated, he might be at home for long periods, intermittently discharging tubercle bacilli, although in general observing hygienic precautions to the best of his ability. Such a patient, in the days before chemotherapy, might not live very long and his infectivity might therefore have a short range in time in contrast to the situation today.

It is not possible to say how many of the total group of children experienced continual and prolonged exposure to this sort of hazard in the home; probably the proportion was not large nor the continuation long. It is therefore all the more striking to see in table 1 that 24 of the total group of children who developed pulmonary tuberculosis were initially in contact with a patient with infectious sputum and that in most cases contact was maintained, more or less unbroken, for several years. Fifteen of the 24 were actually still in contact at the time of, or within two years of, the first detection of their pulmonary tuberculosis; earlier detection might have added 6 more cases. There were, in fact, only 3 cases in this group in which pulmonary tuberculosis developed later than two years after breaking of contact, the chest being roentgenographically normal meanwhile.

In all 24 cases the contact was within the household and, in all except one, was with a close family relation.

Discussion

Of the people who attended the Ealing Chest Clinic in childhood, those who later developed pulmonary tuberculosis may be divided into two groups: those already infected when first seen at the clinic and known, or judged to be, tuberculin positive; and those not yet infected.

The latter, a much smaller group, in general, did not come from tuberculous families and had no known contact with the disease. Casual contact in later years has presumably led to a primary infection which passed, more or less directly, into progressive pulmonary tuberculosis. This accident of contact may have been no greater than in the population at large. The factors determining subsequent events remain unknown.

This group may therefore be regarded as a control series in relation to the much larger proportion of patients already infected before puberty. In these latter patients, the magnitude of their primary infection seems not to have influenced the later development of pulmonary tuberculosis. The presence of a massive primary complex appeared to carry no additional risk, except perhaps when a stage of exudative pleurisy had been reached.

Irrespective of the lapse of time from the date of primary infection, incipient pulmonary tuberculosis commonly became apparent between the ages of fourteen and seventeen. In a high proportion, however, the primary infection had taken place in later childhood, often after the age of twelve. Girls outnumbered boys by four to one. Lincoln (5) and Grzybowski (6) have made similar observations.

Concerning the roentgenographic appearances and later progress of the tuberculous lesions, no special characteristics can be recorded. That the lesions could, in most instances, have been derived from the original primary complex is suggested by the 3 cases of late local recurrence at or near an earlier primary focus. How such a lesion can progress into chronic pulmonary tuberculosis has been well shown by Schwartz (7).

One other fact must, however, be considered. There was a history of contact with a source of tuberculous infection in a high proportion of cases and in practically all patients who were originally tuberculin positive. Moreover, in almost every one of these cases the contact was maintained up to, or very nearly up to, the discovery of pulmonary tuberculosis.

That this characteristic usually included a family history of the disease, and therefore the possibility of a genetic predisposition, cannot lightly be forgotten. Puffer and co-workers (8), for example, have shown that such a factor can operate even in the absence of actual household contact. But many hundreds of children with similar familial tuberculosis in this series have passed safely through the dangerous years of adolescence, as have those with initial, but not continued, exposure to infection. Heredity being equal, it is postpuberty exposure which appears to have been decisive. The experience of Israel and DeLien (9) with rather limited material led them to a like conclusion.

There is a formidable body of opinion, well summarized by Medlar (10), denying the possibility of exogenous superinfection, that is to say, further infection by tubercle bacilli from without, in the continued presence of unhealed primary disease, as evidenced by a tuberculin positive state. Many fieldworkers have been unable to agree to this. The waxing and waning of tuberculin sensitivity in naturally infected persons observed, for example, by Aronson (11) and often seen in hospital nurses (12) seems to be influenced by their exposure to further

infective sources. In a similar way, tuberculin sensitivity induced by BCG vaccination is thought to fade less quickly in an environment of natural infection (13). Only true superinfection could account for such observations.

The clinical results of such an assault can, as a rule, only be inferred. That it may be a real hazard is suggested by Canetti and Robert (14), who followed a group of medical students through their later careers after treatment for pulmonary tuberculosis. After making due allowance for other differences, the rate of breakdown was found significantly higher in those who worked in sanatoriums than in those who did general medical work. Katz and associates (15) found that mental hospital patients with quiescent pulmonary tuberculosis had relapses more often when nursed among infectious cases of tuberculosis than in other parts of the hospital.

That tubercle bacilli may enter and grow within a host already harboring a different strain is suggested by five cases described by Edwards (16) of patients admitted to the hospital with bovine tubercle bacilli in their sputum, who apparently became infected by the human tubercle bacilli of fellow patients. Other clinical evidence of true superinfection is afforded by cases in which BCG vaccination has failed to prevent chronic pulmonary tuberculosis, as in the series reported by the Medical Research Council (17).

If the fact of this phenomenon is accepted, one can only speculate as to its pathogenesis. Exogenous bacilli may themselves set up focal disease. It seems just as likely that their entry is itself abortive, but that a Koch phenomenon is induced thereby, with reactivation of the dormant primary lesions. The association of puberty and its hormonal disturbances, and the predominance of "femaleness," could well be imagined as contributory factors.

The abrupt cessation of new cases in this series three years ago coincides with the development, at about that time, of the full use of effective chemotherapy in pulmonary tuberculosis generally. Only by 1953 were chronic advanced cases being rendered noninfectious on a wide scale, and relapse in those discharged from the hospital becoming subjected to better control. It is impossible not to regard this coincidence as significant.

New patterns of tuberculosis epidemiology are doubtless now in evolution. The series here described may not be repeated in the future, with chronic sources of infection no longer in the home. It is only a decade since Myers (18) remarked: "The medical profession is seriously in need of a criterion by which one can determine whether a given tuberculin reactor will ever develop clinical tuberculosis." Chemotherapy for the primary infection may be one answer to this. Perhaps no less important is protection from further massive or repeated reinfection. It is believed that this latter goal is within reach of becoming assured.

Summary

A group of children originally studied during 1942–1945 has been followed to the present. The development of chronic pulmonary tuberculosis has been observed in 31 cases. An analysis is presented of the characteristics of this disease and the factors leading to its occurrence.

Sumario

Tuberculosis Pulmonar Aparecida en Personas Observadas desde la Infancia

Un grupo de niños estudiados primitivamente durante el período de 1942–1945 ha sido mantenido en observación hasta ahora. En 31 casos se ha observado la aparición de tuberculosis pulmonar crónica. Preséntase un análisis de las características de los factores que conducen a su aparición.

Resume

Développement de la tuberculose pulmonaire chez de sujets observés depuis l'enfance

Un groupe d'enfants étudié pour la première fois au cours des années 1942–1945, a été surveillé jusqu'à maintenant. Le développement de la tuberculose pulmonaire chronique a été observé dans 31 cas. Les caractéristiques de cette affection et des facteurs l'ayant proroquée sont étudiés.

REFERENCES

(1) Thompson, B. C.: Primary tuberculous infection in childhood: Incidence, morbidity and mortality in an urban population, Arch. Dis. Childhood, 1947, *22*, 1.

(2) Thompson, B. C.: Discussion on the fate of the tuberculous primary complex, Proc. Roy. Soc. Med., 1952, *45*, 741.

(3) Thompson, B. C.: Calcified foci within the thorax of English children, Thorax, 1948, *3*, 43.

(4) Thompson, B. C.: Studies in primary pleurisy with effusion, Brit. M. J., 1949, *2*, 841.

(5) Lincoln, E. M.: The value of follow-up studies of children with primary tuberculosis, Am. Rev. Tuberc., 1951, *64*, 499.

(6) Grzybowski, S.: Radiology in childhood tuberculosis, Tubercle, 1954, *35*, 113.

(7) Schwartz, P.: The role of the lymphatics in the development of bronchogenic tuberculosis, Am. Rev. Tuberc., 1953, *67*, 440.

(8) Puffer, R. R., Dillon, A., Gass, R. S., and Hutcheson, R. H.: Tuberculosis attack and death rates of household associates: Influence of age, sex, race, and relationship, Am. Rev. Tuberc., 1952, *65*, 111.

(9) Israel, H. L., and De Lien, H.: The relation of childhood infection to the development of tuberculosis in early adult life, Am. J. Pub. Health, 1942, *32*, 1146.

(10) Medlar, E. M.: The behavior of pulmonary tuberculous lesions, Am. Rev. Tuberc., 1955, *71* (Supplement, p. 220).

(11) Aronson, J. D.: The fluctuation of the tuberculin reaction in different geographic areas and its relationship to resistance, Am. Rev. Tuberc., 1951, *63*, 121.

(12) Thompson, B. C.: Tuberculosis in nurses in a New Zealand hospital, Tubercle, 1949, *30*, 155.

(13) Wallgren, A.: Calmette vaccination in Sweden, Swedish Nat. A. Tuberc., 1947, p. 23.

(14) Canetti, G., and Robert, M.: La reinfection exogene chez le tuberculeux gueri, Rev. de la tuberc., 1950, *14*, 451.

(15) Katz, J., Kunofsky, S., and Locke, B.: Reactivation of inactive pulmonary tuberculosis, Am. Rev. Tuberc., 1956, *73*, 31.

(16) Edwards, P. W.: Personal communication.

(17) Medical Research Council: B.C.G. and vole bacillus vaccines in the prevention of tuberculosis in adolescents, Brit. M. J., 1956, No. 4964, 413.

(18) Myers, J. A., Harrington, F. E., and Garcia Suarez, E.: The importance of detecting tuberculosis in children, J.A.M.A., 1945, *128*, 852.

SKELETAL TUBERCULOSIS IN CHILDREN TREATED FOR PRIMARY AND MILIARY TUBERCULOSIS[1]

LILLIAN MILGRAM

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Introduction

Prior to chemotherapy, tuberculosis of the bone was a frequent complication of primary tuberculosis of childhood. In a 1946–1950 series of 313 infected children less than two years old studied by Miller and McDougall, 19 per cent had bone or joint tuberculosis when first seen or developed it under observation (1*a*). That series contained asymptomatic children with normal chest roentgenograms as well as sick children.

Since most cases of bone tuberculosis occur early in the course of the disease (1*b*), it is now possible to judge the effect of therapy on the incidence of the condition.

An investigation was undertaken of bone and joint complications of children with primary tuberculosis who were followed for at least two years after the tuberculosis was diagnosed. Of special interest were those with miliary tuberculosis, as there is pathologic, clinical, and experimental evidence for the presence of extensive bone infection in that condition when untreated.

Plan of Investigation

Clinical material: The occurrence of bone lesions was determined in children with primary tuberculosis first seen in 1947–1953 (*Group I*), and the incidence in patients treated with streptomycin or isoniazid was compared with that in untreated patients. Those with miliary tuberculosis complicating primary tuberculosis were considered separately. In addition to these children admitted in 1947–1953, patients from two earlier periods were studied for the occurrence of skeletal tuberculosis. One group (*Group II*) was made up of those with miliary tuberculosis (complicating primary tuberculosis) and was treated with a sulfone alone in 1944–1946, just before streptomycin was used. A yet older group (*Group III*) consisted of patients with primary tuberculosis admitted in 1930–1940 who, since they preceded the discovery of specific drugs, form an additional untreated series.

Definition of primary tuberculosis: Children were considered to have primary pulmonary tuberculosis on roentgenographic examination and were included in these study groups since 1930 if they satisfied the following criteria.

Only those who showed roentgenographic changes thought to represent uncalcified, and presumably fresh, primary tuberculosis were included. They were included even when, as happened in a few cases, the history pointed to an infection of longer duration. Excluded

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were children with positive tuberculin tests but normal roentgenograms, patients with primary lesions showing calcification; children with evidence of "reinfection," i.e., chronic pulmonary tuberculosis; and those with other tuberculous lesions, e.g., meningitis, pleurisy, and cervical adenitis, if there was no evidence of the primary node involvement.

Time of recognition of bone lesion: In order to discern a possible preventive effect of drug treatment, lesions of the bone were distinguished according to whether they were recognized (*1*) on admission or in the two weeks following admission to the hospital, so that there was no doubt that the manifestations preceded admission; or (*2*) later—and in the case of the treated—after treatment was begun. It is with this second group that the present study is principally concerned, with particular reference to whether skeletal tuberculosis appeared during therapy or after its completion.

Group I. Study Group

The recent group consisted of 554 children with primary tuberculosis first seen in the outpatient clinic or in the hospital in the period between January 1, 1947, and December 31, 1953, and aged twelve years or less when first seen. Of these, 206 received chemotherapy (for at least one month) and 348 were untreated. Thirty-five of the treated patients also had miliary tuberculosis. The *survivors* of the treated group were compared with the untreated group for the incidence of osseous tuberculosis after admission. (Mastoiditis was not considered.)

Patients Who Received Antimicrobial Treatment

During the years 1947–1951, indications for treatment with streptomycin (given with sulfone (Promizole®) or PAS) changed only slightly, and changes were made principally in the length of therapy with streptomycin (2, 3). With few exceptions, e.g., presumptive bronchogenic spread, enteritis, peritonitis, and mastoiditis, only those children received antimicrobial therapy whose condition made treatment "mandatory" because of the high mortality of the complication (miliary tuberculosis, meningitis, excavating primary disease, and chronic hematogenous tuberculosis). The only large group added was a series of patients with bronchoscopic evidence of endobronchial disease. The study was begun in April, 1948, and continued throughout the entire period. The purpose of this study was to determine the effect of the drugs used on visible endobronchial lesions. There was no concurrent untreated control group with endobronchial disease.

Exception was also made (in a somewhat greater number toward the end of the period studied) in treating a few children with bone tuberculosis, with cervical adenitis, with protracted fever, and with primary tuberculosis of the skin and regional nodes. Brief treatment was given as well to close draining sinuses and to "cover" operative procedures such as tonsillectomy or excision of cervical nodes.

When isoniazid was introduced in 1952, indications for therapy were extended. For the first time some children with less severe, uncomplicated primary tuberculosis—even without visible endobronchial disease—received antimicrobial therapy. In July, 1952, another study group (4) was started, a "primary series" (with untreated concurrent controls) who received isoniazid for one year. The purpose of this study was to determine whether isoniazid protected against meningitis. During 1953 the routine treatment of pleurisy by isoniazid was started in children of seven years or older.

Of the 154 surviving treated patients with primary pulmonary tuberculosis (including all complications except miliary and chronic pulmonary tuberculosis), 89 received streptomycin; 16, both isoniazid and streptomycin; and 49, isoniazid

without streptomycin. For the 30 surviving patients with miliary tuberculosis, these figures were: 22 received streptomycin alone; 7, isoniazid-streptomycin; and one, isoniazid alone (table 1).

Patients who did not receive antimicrobial treatment: It appears from the above analysis of the conditions for which treatment was given that, even aside from the separately considered patients with miliary tuberculosis, the treated group differs from the untreated. The latter is not strictly a control group; it is difficult to say whether it has been "weighted" with patients more likely or less likely to develop tuberculosis of the bone. It consisted of all patients who did not satisfy *changing indications* for antimicrobial therapy. Among the treated are: all children with meningitis (without miliary tuberculosis); all those with primary lesions considered to be locally progressive with excavation; those with endobronchial disease diagnosed during part of the period (without controls); those with primary tuberculosis during a study period (with controls which are included among the untreated cases); most children who had two or more extrapulmonary foci; and some of the children not clearly in these categories who were sick with high or prolonged fever or with marked respiratory obstruction.

TABLE 1

TREATMENT OF SURVIVING PATIENTS WITH PRIMARY TUBERCULOSIS (INCLUDING COMPLICATIONS OTHER THAN MILIARY TUBERCULOSIS) AND PATIENTS WITH MILIARY TUBERCULOSIS

	Streptomycin (with Sulfone or PAS)		Streptomycin and Isoniazid (with Sulfone or PAS)		Isoniazid (with Sulfone or PAS)	
	Primary Tuberculosis	Miliary Tuberculosis	Primary Tuberculosis	Miliary Tuberculosis	Primary Tuberculosis	Miliary Tuberculosis
1947	12	4	—	—	—	—
1948	23	3	—	—	—	—
1949	17	8	—	—	—	—
1950	18	4	—	—	—	—
1951	17	3	2	0	0	0
1952	2	0	6	2	22	0
1953	0	0	8	5	27	1
Total	89	22	16	7	49	1

TABLE 2

SURVIVING PATIENTS OF THOSE ADMITTED, 1947–1953

	1947	1948	1949	1950	1951	1952	1953	Total
Untreated primary pulmonary tuberculosis	72	62	63	54	32	38	22	343
Treated for primary pulmonary tuberculosis or complications other than miliary tuberculosis	12	23	17	18	19	30	35	154
Total primary pulmonary tuberculosis	84	85	80	72	51	68	57	497
Treated for miliary tuberculosis	4	3	8	4	3	2	6	30

The changes in therapeutic and investigative indications are shown by the increasing proportion of children who received therapy; for instance, in 1947, the ratio of untreated to treated patients was 72:12, while by 1953 this ratio was reversed to 22:35 (table 2).

Group II. Sulfone Treated

The sulfone-treated series consisted of children admitted consecutively with primary tuberculosis complicated by acute generalized miliary tuberculosis who were treated with sulfone during 1944–1946, up to the advent of streptomycin therapy.

Group III. Pre-Chemotherapy

The children in this group were admitted consecutively (1930–1940)[8] with primary tuberculosis defined exactly as in the 1947–1953 patients. Those with miliary tuberculosis are not considered separately; instead, separate consideration is given to those who died in three months or less after admission.

Observations

Bone Lesions in Treated Compared with Untreated Primary Tuberculosis (Group I)

Five hundred and fifty-four children with primary tuberculosis were admitted to the hospital in 1947–1953. Thirty-five also had miliary tuberculosis and are

TABLE 3

Bone Lesions Among Surviving Patients

	Group I, 1947–1953				Group II, 1944–1946 Miliary Tuberculosis Treated with Sulfone		Group III, 1930–1940			
	Primary Tuberculosis Exclusive of Miliary Tuberculosis		Miliary Tuberculosis				Surviving 3 Months		Not Surviving 3 Months	
	Number of Patients (497)	Number of Patients with Bone Lesions	Number of Patients (30)	Number of Patients with Bone Lesions	Number of Patients	Number of Patients with Bone Lesions	Number of Patients	Number of Patients with Bone Lesions	Number of Patients	Number of Patients with Bone Lesions
Bone lesion diagnosed on admission		10		1			513	16	106	3 (1 miliary)
Bone lesion diagnosed after admission										
Treated	154	0	30	5	5	4		—		
Untreated	343	6*						9†		
Total	497	16	30	6	5	4	513	25	106	3

* One patient also had an earlier lesion.

† Two patients also had earlier lesions.

analyzed separately. Of the remaining 519, 348 were untreated and 171 received antimicrobial therapy as described above. The surviving patients are analyzed for incidence of osseous tuberculosis (table 3).

Bone lesions were present on admission in 10 patients. All but 2 of these were treated in accordance with the schedules then current on the orthopedic services to which they were transferred. This usually consisted of the daily administration of streptomycin for three months along with PAS or sulfone. One patient was treated for two months, another for eight months; one was also given isoniazid. Tuberculous disease of the bone subsequently developed in one of the 2 children who had not been given antimicrobial therapy for their bone tuberculosis.

Among the 154 survivors of the treated children[2] (exclusive of those with miliary tuberculosis) to the time of writing, none has developed bone tuberculosis while, among the 343 survivors of the untreated group, 6 presented bone lesions after admission (table 3). These gave their earliest symptoms or signs three weeks, one and one-half months, three months, five months, nine months, and twenty-eight months, respectively, after admission (table 4). In all except *C. V.* there was a delay in making the diagnosis, e.g., in the case of *P. J.* tuberculosis of the knee joint persisted undiagnosed for more than two years.

Bone Lesions in Treated Miliary Tuberculosis (Group I)

Thirty-five patients with miliary tuberculosis were treated, 30 of whom survived. Five of these survivors have presented bone or joint tuberculosis since admission to the hospital (table 5). Two had early lesions of the bone which were diagnosed during treatment. In the remaining 3 patients, the lesions of the spine, hip, and knee, respectively, were diagnosed nine, eleven, and eighteen months after the completion of the streptomycin or isoniazid therapy. However, there were abnormalities in a roentgenogram of the femur and hip joint of patient *D. J.* seventeen months before the diagnosis was made (and soon after streptomycin was first given). Not listed in the table is a sixth patient, treated with sulfone for miliary tuberculosis in March, 1947; he had both tuberculosis of the radius with a draining sinus and thoracic spondylitis when admitted to the hospital. This boy received sulfone alone until he developed meningitis a week after treatment was begun, when streptomycin was added.

Bone Lesions in the Group with Miliary Tuberculosis Treated with Sulfone Alone (Group II)

A study was made of the incidence of bone tuberculosis among the small group of survivors of miliary tuberculosis who were given sulfone alone in the period 1944–1946. Of the 9 patients, there were 5 survivors, and 4 of the survivors developed lesions of the bone (table 3). In one case, two bones were involved. The diagnosis was made during therapy in 2 cases, a third had a lumbar spondylitis which appeared and regressed during therapy but was undiagnosed until years later, and the fourth showed a small asymptomatic rib lesion which was first visible roentgenographically five years after treatment.

[2] The deaths in the treated group were chiefly from meningitis.

TABLE 4

BONE AND JOINT TUBERCULOSIS IN UNTREATED CHILDREN

Patient	Sex	Race or National Origin	Age on Admission	Date of Admission	Other Diagnoses Besides Primary Tuberculosis	Bones Involved	Earliest Symptoms or Roentgenographic Changes	Remarks
P.J.	M	N	1 year 7 months	7/15/47		Spine T10 and T11	Neck stiffness and fever 5 months after admission and unexplained; roentgenograms read as negative at that time but later re-read as showing narrowing of interspace between T9 and T10.	Diagnosed because of kyphosis 9 months after admission, following transfer from Bellevue Hospital. No chemotherapy.
						Knee	History of pain in knee 28 months after first admission, following a fall. First swelling of knee 32 months after first admission.	Diagnosed by biopsy of knee joint capsule after two recurrences of swelling, 60 months after first admission.
R.D.	M	W	1 year 7 months	9/22/47	Endobronchial disease	Spine T10 and T11	Lateral view of roentgenogram showed narrowed interspace and paravertebral infiltration 3 months after admission (retrospective observation).	Noticed 4-1/2 months after admission.
F.S.	F	Puerto Rican	1 year 6 months	9/10/48	Supraclavicular adenitis with draining sinus	Spine L3 and L4	Roentgenogram showed definite narrowing of interspace between L3 and L4, 1-1/2 months after admission (retrospective observation).	Neck infection for 5 months previous to admission. Cervical node sinus treated with streptomycin for 14 days, 3 months after admission. Diagnosed 2 months later, following transfer from Bellevue Hospital. Developed meningitis 3 months after this.
V.H.	M	N	6 years 5 months	4/4/50	Endobronchial disease	Ischium—tuberosity	Diagnosed elsewhere 3 months after admission to Bellevue Hospital.	Intracutaneous tuberculin test (+) 15 months before admission. Diagnosed after abscess presented in thigh 3 months after admission, following transfer from Bellevue Hospital.

L.P.	F	Puerto Rican	4 years 6 months	4/10/50		Hip	Diagnosed elsewhere 9 months after admission to Bellevue Hospital.	Intracutaneous tuberculin test (−) 3 months before admission. Diagnosed following transfer from Bellevue Hospital.
C.V.	F	Puerto Rican	11 years 8 months	7/7/50		Spine T5	Diagnosed elsewhere about 3 weeks after admission.	Intracutaneous tuberculin test (+) 8 months before admission. Diagnosed following transfer from Bellevue Hospital.

TABLE 5

BONE AND JOINT TUBERCULOSIS IN MILIARY TUBERCULOSIS

Patient	Sex	Race or National Origin	Age on Admission	Date of Admission	Other Diagnoses Besides Primary and Miliary Tuberculosis	Treatment	Bones Involved	Earliest Symptoms or Roentgenographic Changes	Remarks
M.S.	M	W	11 months	2/18/49	Endobronchial disease. Peritonitis, February, 1950; meningitis, March, 1950.	DHSM, 0.5 gm. daily for 4 months commencing 2/18/49; sulfone 0.5–3.0 gm. daily for 11 months.	Spine T11 and T12	7/8/49 Roentgenogram, 1 month after stopping SM, showed a narrowed intervertebral space between T11 and T12 (retrospective observation).	Discovered 2/17/50, 9 months after stopping SM, because of stiff posture. Osteolysis of vertebrae with paravertebral shadows. Re-treated.
M.Sp.	F	W	1 year 4 months	7/15/49	Cervical adenitis. Poliomyelitis, August, 1949.	DHSM, later SM, 1.0 gm. daily for 5 months commencing 7/12/49; sulfone, 1.0–3.0 gm. daily for one year followed by PAS for 2 years.	Tibia	Ankle swelling present one week after admission, thought to be tenosynovitis. Roentgenogram 7/26/49 reread retrospectively as showing changes.	Roentgenogram 11/2/49, after 3-1/2 months of SM, showed definite changes in the epiphysis and metaphysis of the tibia as well as soft tissue swelling.
D.J.	M	N	1 year 4 months	2/28/51	Meningitis	SM, 1.0 gm. daily for 6-1/2 months commencing 3/1/51; sulfone 0.5–2.0 gm. daily for 2 years.	Hip	Roentgenogram 3/14/51, 2 weeks after starting SM, re-read retrospectively as showing lack of sharpness of outline of femoral epiphysis and mottling in metaphysis.	Diagnosis complicated by contralateral hemiplegia and not made until 8/21/52, when an abscess presented in the thigh, 11 months after stopping SM and 17 months after changes were already present. Re-treated.

R.O.	M	W	4 months	8/27/52	Probable endobronchitis	SM, 1.0 gm. daily for 4 months commencing 8/27/52; INH, 10-7 mg./kg. daily for 6 months commencing 8/27/52; sulfone, 0.5–2.0 gm. daily for 2 years.	Knee	Limp and night pain November, 1954, 18 months after stopping INH.	Swelling of knee noted. Re-treated with INH 12/22/54 and bone changes absent at first; but by 2/18/55 there were destructive changes in the epiphysis and metaphysis of the femur.
D.M.	M	W	1 year 7 months	11/24/53	Endobronchitis. Bilateral tuberculous otitis media; bilateral cervical adenitis. (+) urine culture for acid-fast bacilli.	INH, 10-7 mg./kg. daily commencing 11/24/53 (continues at time of writing); SM, 1.0 gm. daily for 7-1/2 months; commencing 5/14/54, followed by 1.0 gm. biweekly for 3 months; sulfone 0.5–2.0 gm. daily (continues at time of writing).	Spine T8 and T9	Roentgenogram on admission showed osteolysis of vertebrae and narrowing of intervertebral space.	Roentgenogram observed to show abnormality 2 weeks after starting INH. Treatment continued "in definitely," 2 years 4 months to time of writing.

SM—streptomycin; INH—isoniazid; DHSM—dihydrostreptomycin.

Bone Lesions in Group of Untreated Children, 1930–1940 (Group III)

It is difficult to make a comparison between drug-treated patients with primary tuberculosis and those first seen between 1930 and 1940. There was a high mortality among the latter group. For example, of 619 admissions to the hospital, 106 deaths occurred in the first three months and 136 deaths in the first twelve months. Moreover, it was understandably easy to overlook bone lesions among patients who were very sick and about to die. Finally, in this connection, there is the total absence of survivors of miliary tuberculosis.

The 106 children who died in three months (table 3) are not considered, as they throw little light on the incidence of clinical bone tuberculosis. Three of these children had lesions of the bone. Among the 513 who survived at least three months (and many of these later died of miliary tuberculosis), 16 were admitted with bone tuberculosis. Nine patients developed bone lesions after *admission*, 7 within two years (the others at three years and ten years, respectively; one was a tuberculous lesion of the hip which developed after a fall). Of these seven lesions, two occurred in children who already had other bone tuberculosis. Including these 2 children, a total of 16 were admitted with tuberculous osteoarthritis.

It is noteworthy that of the 26 patients with bone tuberculosis, 13 died. All but one died within two years of admission to the hospital. The last-named child, with the late post-traumatic hip disease mentioned above, died of meningitis after a fusion operation on the hip joint.

This old untreated group perhaps serves as a further "control," since it also provides some measure of expected bone lesions when no specific therapy is given.

Discussion

From the experience presented, it appears that chemotherapy given to children with early primary infection can, to a greater or less extent, prevent tuberculosis of the bone. This reduction in osseous tuberculosis is probably a result of the effect of chemotherapy on very small lesions *already in the bone* when administration of the drug is started.

This hypothesis is supported by a number of clinical, experimental, and pathologic considerations:

Clinical bone tuberculosis occurs "early," usually within the first year after infection (1*b*). Preceding this clinical manifestation, there has been a latent period which varies with the bone involved (5). One of the earliest lesions, dactylitis, may appear in one month; and one of the latest, tuberculosis of the hip, in thirty months after the onset of tuberculosis. The writer has repeatedly had the experience—once the diagnosis of bone disease has been definitely made roentgenographically—of reviewing the patient's previous films and finding that, long before there had been any clinical suspicion, changes were already present (tables 4 and 5). In one child who received only sulfone therapy, roentgenograms revealed an increasing—then a decreasing—lumbar spondylitis which was not apparent clinically and was undiagnosed until after the arrest of the bone disease.

In experimental animal infection, organ implantation occurs very early, after intravenous, intracutaneous, or subcutaneous injection of tubercle bacilli (6–8). By using the method of animal reinoculation, Soltys and Jennings demonstrated the presence of tubercle bacilli in the bone marrow of the guinea pig as early as one hour after subcutaneous inoculation with 0.3 mg. of bacilli of bovine origin. With relatively mild infecting doses, there is evidence that the bacillemia, and thus the likelihood of further organ implantation, may soon cease (9, 10).

Minute foci in some of the organs can become scarred or disappear on therapy, as may be seen in the pathology of treated miliary tuberculosis (11–13). However, in contrast to minute foci, areas of clinical bone disease have been resistant to total eradication by drug treatment alone. This was true of streptomycin therapy (14, 15) and appears to hold for combined isoniazid-streptomycin in clinical and experimental bone infections (16, 17). Barclay and associates (18) have found that isoniazid is poorly distributed into the bones, and one may conjecture that the amount may be adequate for treatment of miliary but not for the larger necrotic foci.

Another factor which may be involved in the clearing of the lesions is their relative freshness. It was noted early by Hinshaw and Feldman (19, 20) that fresh exudative lesions were especially sensitive to streptomycin treatment, and this observation has been repeatedly confirmed in the chemotherapy of tuberculosis (21). In cases of hydrarthrosis of the knee joint treated with streptomycin, Galland (22) found that the ultimate result in terms of function was far better when treatment was started during the first six months of the disease than when therapy was started at a later point. Although nothing has been said here about the spontaneous clearing of latent bone foci, there can be no doubt of its importance and extent. Such natural healing was demonstrated experimentally by Doan and Sabin (23) in the bone marrow of rabbits infected by intravenous injection with lethal doses of bovine bacilli.

At the start of treatment there are miliary bone foci, more or less advanced, and demonstrable in biopsy specimens (24) of accessible bones. At autopsy of patients who died of miliary tuberculosis, all were found by Randerath (25) to have miliary lesions of the bones examined—vertebrae, sternum, ribs, or femur. In some of his cases, the miliary lesions were confluent to a size that could be properly designated "osteitis." The therapy administered to the patients in the present series did not eradicate some of these bone foci, which developed into early clinical bone disease. With streptomycin and isoniazid therapy, fewer such lesions develop than under sulfone therapy.

The bacteremia of miliary tuberculosis (26) is suppressed in successfully treated children. But, as long as late bone and other extrapulmonary tuberculosis occurs, there will be uncertainty (*1*) whether these were due to later post-treatment bacillemia and the formation of new lesions; or (*2*) whether there was long suppression of activity of uneradicated old dormant foci. An example of the latter type of lesion was the single bone-marrow focus found by Doan and Sabin (23) in a rabbit sacrificed after one hundred and twenty days. Steiger (27) among others believed that there is a latent or dormant infection of the tissues, but

stated that he had no evidence whether the tubercle bacilli lie in the tissues and the growth comes later, or whether the tuberculosis progresses more or less slowly from the beginning.

Years ago, Willis (7) compared tuberculosis with syphilis in its early bacteremia and early dissemination into the organs—spleen, bone marrow, and lymph nodes. The similarity seems to hold further in the dormancy of that infection in the bones as well as in other tissues. In syphilis, too, osteitis occurs after trauma. Stokes (28) cites, as typical of a considerable group of reported observations, Landouzy's case of an army officer who, after twenty-five years of latent syphilis, developed an osteitis following a fall from his horse. Here, as in traumatic bone tuberculosis, a latent focus was probably activated by the injury. For syphilis, Stokes mentions Kolle's opinion that there may exist in man, as in rabbits and mice, a carrier state in which there is no reaction against the infection.

Bone lesions have been manifest clinically in 2 patients and diagnosed solely by roentgenography in 2 additional patients among the 5 survivors of miliary tuberculosis treated with a sulfone alone. Of those treated with the more powerful drugs (streptomycin or isoniazid, or both) 5 of 30 survivors of miliary tuberculosis so far have manifested bone or joint tuberculosis. A sixth patient had osteitis before he developed his miliary disease. These are small series; yet it seems clear that these better drugs, even when given for short periods as measured by current standards, prevented much bone disease in the survivors. This presumably resulted from the treatment of the unknown metastases, the preclinical foci of tuberculosis of the bone. Prior to specific therapy, bone lesions were very frequent among those whose miliary tuberculosis ran a long or favorable course. Hoyle and Vaizey (29), in their series of ten cases of "chronic miliary" tuberculosis (three patients treated with gold, two with tuberculin injections for iritis, the rest not specifically treated) found that five had skeletal tuberculosis at the time of miliary tuberculosis, in two of whom the disease of bone was diagnosed after the miliary dissemination was discovered. Eight of van Creveld and Huët's (30) fourteen patients with chronic miliary tuberculosis had skeletal tuberculosis. This also may give some measure of the preventive effect of streptomycin and isoniazid therapy.

However, the experience of the present writer is that treatment of an osteitis which is already advanced may fail to halt its progress. Accordingly, it still remains to be seen how many tuberculous foci in bone which are incompletely healed at the time treatment is stopped are temporarily suppressed only to make their clinical manifestation later, and how many are held in check or healed by the now unaided healing processes of the body.

Summary

A group of children less than thirteen years of age with roentgenographic evidence of primary tuberculosis was followed for two to eight years. During the period of follow-up, skeletal lesions occurred with greatest frequency among the survivors of miliary tuberculosis. The incidence was low among children

treated for indications other than miliary disease, including those treated for skeletal tuberculosis, and was lower than in an untreated group. The complication was less frequent under streptomycin and isoniazid treatment than under treatment by the previously used sulfone, Promizole®.

It is suggested that the bones are infected early in primary tuberculosis and that chemotherapy acts on already established foci. There is evidence that at the beginning of treatment of miliary tuberculosis, there is widespread involvement of the bone marrow. Such foci probably account for the frequency of clinical bone tuberculosis in treated generalized tuberculosis.

Sumario

Complicaciones Oseas en Niños Tratados por Tuberculosis Primaria y Miliar (Granulia)

Un grupo de niños de menos de trece años de edad con signos radiográficos de tuberculosis primaria fué mantenido en observación durante dos a ocho años. Durante este período, hubo lesiones óseas con la mayor frecuencia en los sobrevivientes de granulia. La incidencia fué baja entre los niños tratados por indicaciones distintas de granulia, comprendiendo los tratados por tuberculosis ósea, y siendo menor que en un grupo no tratado. La complicación fué menos frecuente con el tratamiento con estreptomicina e isoniacida que con la sulfona usada anteriormente, Promizol®.

Apúntase que los huesos se infectan tempranamente en la tuberculosis primaria y que la quimioterapia obra sobre focos ya establecidos. Hay datos de que, al comenzar el tratamiento de la granulia, existe invasión generalizada de la médula ósea. Esos focos probablemente explican la frecuencia de la tuberculosis ósea clínica en la tuberculosis generalizada tratada.

Resume

Complications osseuses chez les enfants traités pour tuberculose de primo-infection ou miliaire

Un groupe d'enfants âgés de moins de treize ans présentant des signes radiologiques de tuberculose primaire a été suivi pendant une période de deux à huit ans. Durant la période d'observation, les lésions osseuses apparaissaient avec la fréquence la plus grande parmi ceux ayant survécu à une tuberculose miliaire. L'incidence était basse parmi les enfants traités pour d'autres formes de la tuberculose, y compris ceux traités pour tuberculose osseuse, et elle était plus basse que celle observée dans un groupe non-traité. Cette complication était moins fréquente quand le traitement comprenait la streptomycine et l'isoniazide plutôt que la sulfone, Promizole®, utilisée antérieurement.

L'auteur considère que l'infection de l'os se produit au début de la primo-infection et que la chimiothérapie agit sur les foyers déjà établis. On possède la preuve qu'au début du traitement d'une tuberculose miliaire, la moelle osseuse est déjà largement affectée. Ce sont ces foyers qui sont probablement responsables de la fréquence de la tuberculose osseuse avec manifestations cliniques dans la tuberculose généralisée traitée.

REFERENCES

(1) *a.* Miller, F. J. W., and McDougall, R.: Recognition of tuberculosis in children under 2 years, Brit. M. J., 1954, No. 4892, 846.

b. Wallgren, A., and Lundblom, A.: Ueber den Zeitpunkt des Auftretens der Skelettuberkulose, Tuberkulose, 1935, *15*, 164.

(2) Lincoln, E. M., and Kirmse, T. W.: Chemotherapy of tuberculosis in children, Pediatrics, 1950, *5*, 280.

(3) Lincoln, E. M., and Wilking, V. N.: Specific treatment of children with tuberculosis, A.M.A. J. Dis. Child., 1951, *82*, 655.

(4) Lincoln, E. M., Sewell, E. M., and Anastasiades, A. A.: The treatment of primary tuberculosis in children, Postgrad. Med., 1954, *16*, 422.

(5) Lang, W.: Die Bedeutung der Latenzzeit tuberkulöser Skelettherde für die Begutachtung, Tuberkulosearzt, 1952, *6*, 413.

(6) Krause, A. K.: Studies on tuberculous infection: VI. Tuberculosis in the guinea pig after subcutaneous infection with particular reference to the tracheobronchial lymph nodes, Am. Rev. Tubere., 1920, *4*, 135.

(7) Willis, H. S.: Studies on tuberculous infection: X. The early dissemination of tubercle bacilli after intracutaneous inoculation of guinea pigs of first infection, Am. Rev. Tubere., 1925, *11*, 427.

(8) Soltys, M. A., and Jennings, A. R.: The dissemination of tubercle bacilli in experimental tuberculosis in the guinea pig, Am. Rev. Tubere., 1950, *61*, 399.

(9) Schwabacher, H.: Experimental bacillaemia in guinea-pigs and rabbits, in *Tuberculous Bacillaemia*, by Wilson, G. S., British Medical Research Council Special Report Series, London, 1933, No. 182, Appendix B, p. 112.

(10) Breton, M., Massol, L., and Duhot, E.: Recherche du bacille de Koch dans le sand au cours de l'infection expérimentale du cobaye, Compt. rend. Soc. de biol., 1913, *121*, 792.

(11) Bagenstoss, A. H., Feldman, W. H., and Hinshaw, H. C.: Streptomycin in miliary tuberculosis: Its effect on pathological lesions of generalized miliary tuberculosis in human beings, Am. Rev. Tubere., 1947, *55*, 54.

(12) Flory, C. M., Correll, J. W., Kidd, J. G., Stevenson, L. D., Alvord, E. C., Jr., McDermott, W., and Muschenheim, C.: Modifications of tuberculous lesions in patients treated with streptomycin, Am. Rev. Tubere., 1948, *58*, 421.

(13) Ritchie, G. M., Taylor, R. M., and Dick, J. D.: Effect of streptomycin and isoniazid on miliary tuberculosis and tuberculous meningitis, Lancet, 1953, *2*, 419.

(14) Bosworth, D. M., and Wright, H. A.: Streptomycin in bone and joint disease, J. Bone & Joint Surg., 1952, *34-A*, 255

(15) Ghormley, R. K.: Introductory note to symposium: Streptomycin in treatment of bone and joint tuberculosis, Public Health Service Cooperative Investigation, J. Bone & Joint Surg., 1952, *34-A*, 254.

(16) Sorell, Sorell, Mme., Gerard-Marchant, Galland, and Giret: Tuberculose ostéo-articulaire et I.N.H. pur ou associé (d'après 555 cas traités dans les sanatoriums spécialisés, Rev. de la tubere., 1954, *18*, 26.

(17) Bastos Mora, F., and Portal Llamedo, L.: Experimental studies on the treatment of bone and joint tuberculosis with dihydrostreptomycin and isonicotinic acid hydrazide, J. Bone & Joint Surg., 1955, *37-A*, 156.

(18) Barclay, W. R., Ebert, R. H., LeRoy, G. V., Manthei, R. W., and Roth, L. J.: Distribution and excretion of radioactive isoniazid in tuberculous patients, J.A.M.A., 1953, *151*, 1384.

(19) Hinshaw, H. C., and Feldman, W. H.: Streptomycin in the treatment of clinical tuberculosis: A preliminary report, Proc. Staff Meet., Mayo Clin., 1945, *20*, 313.

(20) Hinshaw, H. C., Feldman, W. H., and Pfuetze, K. H.: Streptomycin in the treatment of clinical tuberculosis, Am. Rev. Tubere., 1946, *54*, 191.

(21) Bernard, E., Kreis, B., and Lotte, A.: Sur l'importance du facteur "lésion récente" dans les effets du traitement de la tuberculose pulmonaire par l'isoniazide, Presse méd., 1953, *61*, 681.

(22) Galland, M.: Le probléme de la tuberculose ostéo-articulaire est-il changé? Bruxelles-méd., 1952, *32*, 2113.

(23) Doan, C. A., and Sabin, F. R.: Local progression with spontaneous regression of tuberculosis in the bone marrow of rabbits correlated with a transitory anemia and leucopenia after intravenous inoculation, J. Exper. Med., 1927, *46*, 315.

(24) SCHLEICHER, E. M.: Miliary tuberculosis of the bone marrow, Am. Rev. Tuberc., 1946, *53*, 115.
(25) RANDERATH, E.: Pathologisch-anatomische Untersuchungen über die Tuberkulose des Knochensystems, Beitr. z. Klin. Tuberk., 1932, *79*, 201.
(26) WILSON, G. S.: Tuberculous Bacillaemia, British Medical Research Council Special Report Series, London, 1933, No. 182.
(27) STEIGER, J.: Zur Klinik der hämatogenen Tuberkulose, Schweiz. med. Wchnschr., 1933, *1*, 310.
(28) STOKES, J. H., BEERMAN, H., AND INGRAHAM, N.: Modern Clinical Syphilology, ed. 3, W. B. Saunders Co., Philadelphia and London, 1944, pp. 11 and 15.
(29) HOYLE, C., AND VAIZEY, M.: Chronic Miliary Tuberculosis, Oxford University Press, London, 1937.
(30) VAN CREVELD, S., AND HUËT, G. J.: Chronic miliary tuberculosis, Acta med. Scandinav., 1943, *113*, 135.

EXPERIENCE WITH THE COURSE AND CHEMOTHERAPY OF CHRONIC PULMONARY HISTOPLASMOSIS[1, 2]

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Introduction

Chronic pulmonary histoplasmosis has been recognized with increasing frequency in recent years and it is of general interest because it simulates other chronic pulmonary diseases (1). Current reports (1–3) show that chronic pulmonary histoplasmosis is the predominant clinical form of the disease in adults. Other clinical forms of histoplasmosis, such as the acute pulmonary disease and the disseminated form, are seen less often. The cases of chronic pulmonary histoplasmosis described below are especially suitable for a clinical description of the chronic form of the disease and for the clinical trial of antimicrobials because the etiology was well established by culture of *Histoplasma capsulatum*.

Clinical Material

During the five years from 1951 to 1956, the etiologic agent *H. capsulatum* was isolated and identified by culture of sputum, bronchial washings, or tissues in 23 cases of chronic pulmonary histoplasmosis. The patients were treated in a Veterans Administration hospital which received patients from the population in the Mississippi Valley. All of the patients were males, thirty-three to seventy-three years of age; only one of the patients was a Negro; a majority (17) were farmers, although a few had some additional trade or business. Of the 6 who were not farmers, 2 were textile workers in garment factories; and there was one case each of hospital fireman, hospital attendant, real estate salesman, and student. Inquiries as to special types of exposure elicited further information in 11 cases. There was information about large-scale chicken farming in 2 cases, repeated unusual exposure to chicken manure in 6 cases, and exposure to pigeons in one case. Dusty old corncribs were the scenes of unusual degrees of exposure to dust in 2 cases. In the remaining 12 cases there was no history of specific exposure. The disease ran a chronic course. The average length of observation after diagnosis was 17.1 months per case. The total known duration in the 23 cases ranged from 17 months to 12½ years, an average of 58.4 months per case.

Specific Diagnostic Methods Employed

Specific culture, serologic, and skin test evidence was essential to the diagnosis of chronic pulmonary histoplasmosis to differentiate the disease from other chronic pulmonary infections, particularly tuberculosis. The sputum was the source of cultures positive for *H. capsulatum* in 19 of the 23 cases, and tissue removed at operation was the source of microorganisms in the 4 remaining cases. Such cultures required from two to four weeks, or longer, for isolation and identification of the microorganism. Initial tentative identification of the etiologic

[1] From the Infectious Disease Section of the Medical Service, Veterans Administration Hospital (Kennedy Division), Memphis, Tennessee.

[2] Presented, in part, before the Medical Session, as part of *Section 5A*, at the annual meeting of the National Tuberculosis Association, New York, New York, May 23, 1956.

agent was therefore made in some cases by other quicker means. In some cases the organism was recognized by direct microscopic examination of the sputum stained with the Romanowsky stain, such as Wilson's or Giemsa.

Microorganisms resembling *H. capsulatum*, sometimes intracellular (figure 1) and sometimes larger forms in extracellular positions (figure 2), were observed on direct examination of the sputum in 7 cases.

In 3 cases the initial evidence of histoplasmosis was obtained by serologic tests. Twenty-two of 23 patients in whom such tests were performed had titers of 1:16, or greater, in a complement-fixation test using the yeast phase antigen. The diagnosis of histoplasmosis could have been tentatively recognized in all cases if serologic tests had been carried out at the time of the initial investigation. As the result of this experience and other experience reported elsewhere (4), it is believed that serologic tests may be useful for survey purposes to select cases for further fungal cultures and for thorough clinical study for histoplasmosis. All of the patients had positive skin test reactions with standardized histoplasmin. Such positive tests were of little diagnostic aid, however, because the incidence of positive histoplasmin skin tests is high in patients from many parts of the area from which cases were received. In patients from other areas where the incidence of positive reactions to histoplasmin skin test is low, the occurrence of positive reactions might be of greater significance. Negative histoplasmin skin tests, on the other hand, were of value in excluding the diagnosis of histoplasmosis. Diagnosis was also aided by thorough studies for tuberculosis when they ruled out active tuberculous infection. For this purpose, the negative Mantoux test in 2 of the cases was of the greatest significance.

Symptoms

Most of the patients sought medical examination after symptoms became obvious. The relative frequency of the principal symptoms is shown in table 1. The eight most frequent symptoms were cough, mucopurulent sputum, weight loss, chest pain, weakness or fatigue, fever, dyspnea on exertion, and bloody sputum, in that order.

Early Manifestations

In 2 employees of the hospital, a series of roentgenograms before and after the appearance of the first pulmonary lesion fixed the date of onset of pulmonary lesions and brought out the insidious nature of the onset. The following brief history and roentgenograms of one of these 2 patients illustrate the early course of chronic pulmonary histoplasmosis.

Case 1. D. W. was a 37-year-old white male hospital fireman. He noted pleural pain and symptoms of influenza twenty months before hospital admission. Subsequent examination of the roentgenogram taken at the onset showed the development of a very small infiltration, but a series of roentgenograms taken annually before the onset was normal. A slight increase in the infiltration (figure 3) appeared thirteen months after the onset. At the time of admission, twenty months after onset, an annular shadow 3 cm. in diameter with a thin wall (figure 4) was seen at the second left anterior rib level with infiltrations extending in a linear manner to the hilum. Calcified nodules were present in the hilum and in the lung

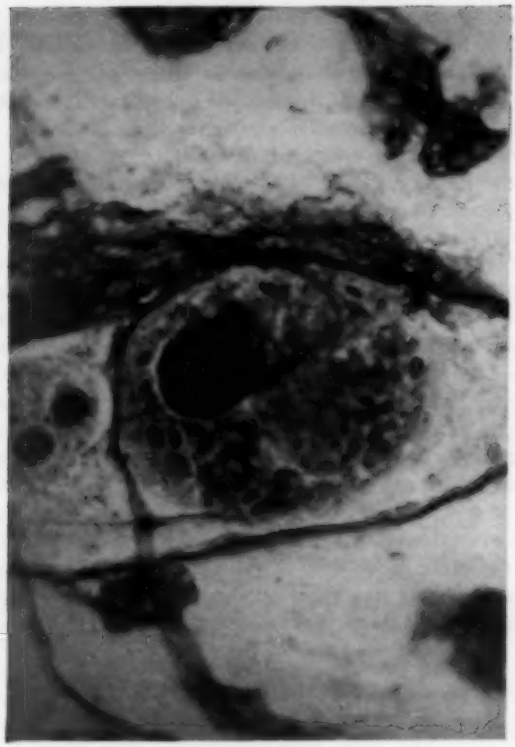


FIG. 1. Intracellular microorganisms resembling *H. capsulatum* in the sputum of a patient with chronic pulmonary histoplasmosis. (× 900 Wilson's stain)

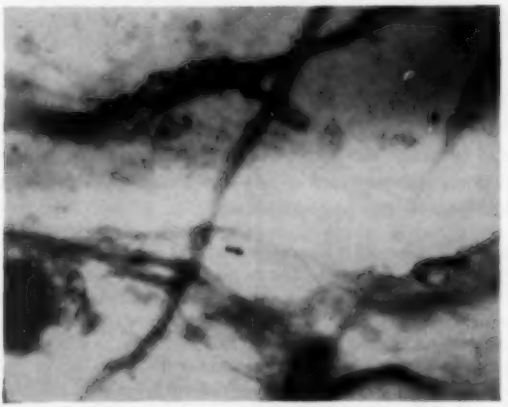


FIG. 2. Extracellular microorganisms resembling *H. capsulatum* in the sputum of a patient with chronic pulmonary histoplasmosis. (× 900 Giemsa stain; enlarged four times)

TABLE 1

FREQUENCY OF CERTAIN SYMPTOMS AT ONSET AND AT HOSPITAL ADMISSION IN 23 CASES OF CHRONIC PULMONARY HISTOPLASMOSIS

Symptoms	Number of Cases	Symptoms	Number of Cases
Cough	22	Bloody sputum	4
Mucopurulent sputum	14	Pneumonia (history of)	3
Weight loss	9	Cold, "flu" (history of)	2
Chest pain	9	Generalized aches	1
Weakness, fatigue	9	Jaundice	1
Fever	7	Ulcer of tongue	1
Dyspnea on exertion	7	Wheezing	1

Case 1

FIG. 3 (Left). Roentgenogram showing minimal pulmonary infiltration thirteen months after onset of disease.

FIG. 4 (Right). Roentgenogram showing annular thin-walled shadow on hospital admission twenty months after onset of disease.

parenchyma. Past history disclosed exposure to chicken manure and to turkeys. The patient had had a duodenal ulcer for ten years. He had no fever and no physical signs of pulmonary infiltration. A serologic test for histoplasmosis was positive in a dilution of 1:16. Histoplasmin and Mantoux skin tests (PPD 0.0002) were positive. The erythrocyte sedimentation rate was accelerated (Wintrobe method). Neither tubercle bacilli nor fungi were found in the sputum culture. Resection of the posterior apical segment of the left upper lobe was performed two months after admission. Cultures of bronchial washings taken two months before operation, and from the pulmonary cavities in the resected lung, showed *H. capsulatum*. Subsequent sputum specimens were repeatedly positive for *H. capsulatum* both by culture and by direct microscopy.

The postoperative course was stormy, and a recurrence of the pulmonary infiltration and cavitation developed and stabilized in the left upper lobe during the following year. The patient was treated with beta-di-ethyl-aminoethyl-fencholate[2] without effect. Following segmental resection and thoracoplasty, first stage, and twelve months of modified bed rest in the hospital, the patient was able to return to less strenuous work than his previous job twenty-six months after admission.

[2] MRD-112, Merrell research drug.

This patient's story illustrates the insidious nature of the onset of chronic pulmonary histoplasmosis and shows further that large thin-walled shadows occur as a relatively early manifestation of chronic pulmonary histoplasmosis. The outcome was satisfactory but the pulmonary lesion persisted and work tolerance was impaired.

Late Manifestations

The fully developed clinical picture of histoplasmosis is illustrated by the following case:

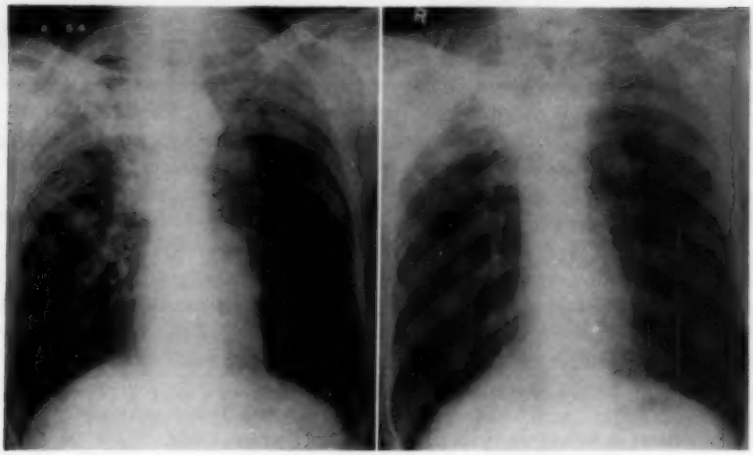
Case 2. G. W. W., a 63-year-old white male Missouri farmer, was admitted to the hospital because of chronic cough beginning thirteen months before admission following an attack of pneumonia. He gave a history of cleaning his chicken house about once a month.

Physical examination showed that his general habitus was similar to that which characterizes the majority of patients with chronic pulmonary histoplasmosis. He was thin, with a drawn expression to his facies, and was easily fatigued and slow in thought and movement. A roentgenogram obtained three months after onset showed bilateral patchy pneumonia in both upper lung fields (figure 5). A roentgenogram of the chest on hospital admission fifteen months after onset of the disease (figure 6) showed bilateral apical fibrous lesions with multiple cavities 1 to 3 cm. in diameter; calcified nodules were present in both lung fields, both hila, and in the spleen. The hilar structures were displaced upward bilaterally and the trachea was displaced to the right. Increased radiolucency was noted in both lung fields. *H. capsulatum* was found persistently in the sputum by direct examination and by culture. Serologic tests were positive with *H. capsulatum* yeast-phase antigen in high titer, from 1:128 to 1:256. Histoplasmin and Mantoux (PPD 0.005) skin tests were positive. The erythrocyte sedimentation rate was accelerated. The albumin-globulin ratio was reversed. Eighteen sputum specimens were negative for acid-fast bacilli; nine gastric washings were negative on culture for tubercle bacilli of human origin, and one was positive and considered to be an error. The patient's general condition and pulmonary lesion remained unchanged during sixteen months of observation, mostly at modified bed rest in the hospital. He was treated with nystatin (Mycostatin®) intramuscularly, cycloserine (Seromycin®) orally, and beta-di-ethyl-aminoethyl-fencholate intravenously without effect.

This case illustrates the stable nature of the pulmonary lesions, the presence of marked fibrosis and cavitation, and the tendency to prolonged disability without apparent progression.

Course and Outcome

The course of the disease was chronic and fairly uniform. One patient is apparently well twenty-two months after diagnosis following surgical removal of the whole area of pathologic tissue by right upper lobectomy. In 5 cases substantial improvement occurred, both in pulmonary lesions and in symptoms, but certain lesions persisted. Fourteen of the patients remained unimproved when last seen after a period of observation averaging seventeen months per case. Death occurred in 4 cases. Pulmonary complications were present in 2 patients who died in the hospital and were examined post mortem: pulmonary emphysema, pulmonary fibrosis, and cor pulmonale in one case; and pulmonary emphysema, pulmonary fibrosis, pneumatocele, chronic pulmonary arterial thrombosis, and abscess due to a drug-resistant *Micrococcus pyogenes* in the other case. One of the 2 patients who died outside the hospital had tuberculosis. Death was not associated in this case with dissemination of histoplasmosis to other organs.



Case 2

FIG. 5 (Left). Roentgenogram showing bilateral multiple pulmonary infiltrates three months after onset of disease.

FIG. 6 (Right). Roentgenogram showing bilateral infiltrations with multiple cavities and displacement of trachea and hilum structures fifteen months after onset of disease.

DISCUSSION

A thumbnail description of chronic pulmonary histoplasmosis is offered, based upon these 23 cases in which the diagnosis was established with the aid of positive cultures for *H. capsulatum*. The disease occurred most often among farmers. It began insidiously, or with symptoms of pneumonia. The course was chronic, with chronic cough, mucopurulent sputum, weight loss, chest pain, weakness and fatigue as the principal symptoms. Marked fibrosis of the pulmonary lesions led to displacement of the trachea and hilar structures toward the areas of maximal involvement. Cavities that were sometimes large and thin walled and often multiple and small in size were usually demonstrable. Acute complicating pulmonary infections associated with the presence of bacteria, such as *Klebsiella pneumoniae* and *M. pyogenes*, were observed. Pulmonary tuberculosis was present in one case. The most frequent complications were emphysema and fibrosis associated with dyspnea on exertion. The outcome was unfavorable in 4 cases, unchanged in 14 cases, and improved in 6 with current modes of therapy, following an average duration of about five years.

Surgery and antimicrobial therapy were used systematically in attempts to influence favorably the otherwise fairly uniform course of the disease (table 2).

The first chemotherapeutic agent to be used was ethyl vanillate (5), a higher phenol manufactured from paper mill waste which has been approved for therapeutic use by the Food and Drug Administration. It is fungistatic for *H. capsulatum* in concentrations of 0.2 to 0.3 mg. per ml., and the LD_{50} dose orally in mice is 1,600 mg. per kg. It has a vile taste and causes prompt gastrointestinal distress because of its irritative qualities, and yet it needs to be administered in

TABLE 2

RESULTS OF THERAPY IN 23 CASES OF CHRONIC PULMONARY HISTOPLASMOSIS AND 2 CASES OF DISSEMINATED HISTOPLASMOSIS*

Therapy	Number of Cases	Number of Cases		
		Unimproved	Improved	Cured
Excision	8	4†	3	1
Ethyl vanillate	5	4†	1 (Disseminated)	0
Beta-di-ethyl-aminoethyl-fencholate	7	5	2 (1 Case disseminated)	0
Nystatin	4	4	0	0
Others	3	3†	0	0
No special therapy	5	5†	0	0

* Several patients received more than one special therapy; a total of 18 patients had special therapy.

† One patient in each category died and a total of 2 patients died.

large doses, as much as 50 gm. per day orally. The dosage should be controlled by daily determinations of the blood concentration so that it can be kept below 30 mg. per 100 ml. This drug was administered in 4 cases of chronic pulmonary histoplasmosis, and no favorable therapeutic effect was noted. One patient with disseminated histoplasmosis improved sufficiently so that no signs of active histoplasmosis remained. Cortico-adrenal insufficiency, which appeared before the ethyl vanillate therapy, persisted and led ultimately to a fatal outcome four years later. The only lesion of histoplasmosis was found in the adrenal glands. This one partially favorable result was consistent with the favorable reports of others, in that improvement occurred in a case with disseminated lesions. The results of the group of cases, however, do not furnish encouragement for further use in chronic pulmonary histoplasmosis.

Beta-di-ethyl-aminoethyl-fencholate (6), an experimental therapeutic agent for the mycoses, was administered in 7 cases. This chemical is related to other compounds with antihistaminic and sedative action and has a sedative effect. Antifungal action can be demonstrated *in vitro* in a concentration of 0.1 mg. per ml. The LD_{50} intravenous toxic dose for mice is approximately 72 mg. per kg. This drug was given intravenously in doses of 150 to 600 mg. per day for periods of one or two months. In 5 cases of chronic pulmonary histoplasmosis of average severity, no favorable effect was noted. One patient with chronic pulmonary histoplasmosis of minimal severity who was seen relatively early was improved following therapy. Another patient with disseminated histoplasmosis was markedly improved following therapy. These two favorable results reveal the possibility that beta-di-ethyl-aminoethyl-fencholate may have some favorable effect on some cases of minimal disease or disseminated lesions, but it is apparent that no definitely favorable results can be expected in the well-developed case of chronic pulmonary histoplasmosis.

Nystatin (7) is an experimental antifungal antimicrobial with marked *in vitro* fungistatic action in concentrations of 1 to 6 γ per ml. and an LD_{50} toxic dose

intravenously in mice of from 28 to 61 mg. per kg. This antimicrobial was given intramuscularly and produced toxic effects constantly during the administration, consisting of fever and chills, pain at the site of injection, and occasional abscess formation with temporary draining sinus. No favorable therapeutic effects were noted in 4 cases of chronic pulmonary histoplasmosis.

Actidione, 2-aminostilbamidine, and cycloserine were also administered in one case each of chronic pulmonary histoplasmosis without favorable therapeutic effect. The results of surgical therapy, which was used in 8 cases, will be reported by Hughes and co-workers. Patients given no specific therapy are also listed in table 2. Five patients with chronic pulmonary histoplasmosis treated without specific therapy remained unimproved during periods of modified bed rest in the hospital.

A general conclusion was reached that more potent and less toxic antifungal agents were needed for adequate favorable therapeutic effects in chronic pulmonary histoplasmosis.

Summary

The clinical manifestations in 23 cases of chronic pulmonary histoplasmosis confirmed by cultures of *Histoplasma capsulatum* are described. One case each with gradual onset and with advanced pulmonary changes, respectively, is cited.

Special therapy was administered whenever available: Ethyl vanillate was administered in 5 cases; beta-di-ethyl-aminoethyl-fencholate (MRD-112) in 7 cases; nystatin in 4 cases; and actidione, 2-aminostilbamidine, and cycloserine in one case each. In chronic pulmonary histoplasmosis, definite improvement was noted in only one relatively mild case, following beta-di-ethyl-aminoethyl-fencholate therapy, of a total of 13 cases treated with various agents. Two cases of disseminated histoplasmosis were both arrested following treatment: in the one case with ethyl vanillate, and in the other case with beta-di-ethyl-aminoethyl-fencholate. Toxic symptoms limited the use of all of these agents in varying degrees. A general conclusion was reached that more potent and less toxic antifungal agents were needed for adequate favorable therapeutic effects in chronic pulmonary histoplasmosis. Further trial of these agents in the disseminated form of the disease may be of value.

Sumario

Observaciones Relativas a la Evolución, la Quimioterapia y la Terapéutica Antimicrobiana de la Histoplasmosis Pulmonar Crónica

Describense las manifestaciones clínicas observadas en 23 casos de histoplasmosis pulmonar crónica, confirmados por cultivos del *Histoplasma capsulatum*. Citanse 2 casos, uno de iniciación paulatina y uno con alteraciones pulmonares avanzadas.

Se administró terapéutica especializada, siempre que la había: en 5 casos se aplicó el vainillato etílico, MRD-112 en 7, la micostatina en 4 y actidiona, 2-aminostilbamidina y cicloserina en un caso cada una. En la histoplasmosis pulmonar crónica, de un total de 13 casos tratados con varios agentes, sólo se notó mejoría bien definida en un caso relativamente leve, a continuación de la MRD-112-terapia. Dos casos de histoplasmosis diseminada se estacionaron a continuación del tratamiento, uno con vainillato etílico y el otro con MRD-112. Síntomas tóxicos limitaron en mayor o menor grado el uso de todos estos agentes. Se alcanzó la conclusión general de que se necesitaban agentes antimicóticos más potentes

y menos tóxicos para obtener efectos terapéuticos adecuados y favorables en la histoplasmosis pulmonar crónica. Pruebas ulteriores de estos agentes en la forma diseminada de la dolencia pueden resultar valiosas.

Resume

Expérience acquise sur l'évolution, la chimiothérapie et la thérapeutique antibactérienne de l'histoplasmose pulmonaire chronique

Description des manifestations cliniques dans 23 cas d'histoplasmose confirmée par la présence de *Histoplasma capsulatum* dans les cultures. Un cas s'étant développé graduellement et un cas avec lésions pulmonaires avancées sont exposés.

Chaque fois que cela a été possible, un traitement spécial a été administré: le vanillate d'éthyle a été administré dans 5 cas; MRD-112, dans 7 cas, la mycostatine, dans 4 cas et l'actidone, la 2-aminostilbamine et la cyclosérine, chacune dans un cas. Dans l'histoplasmose pulmonaire chronique, sur 13 cas traités par divers agents, il a été observé une amélioration indiscutable dans un seul cas, qui était relativement léger, à la suite du traitement par MRD-112. Deux cas d'histoplasmose disséminée ont été stabilisés, dans un cas après traitement par le vanillate d'éthyle et dans l'autre par le MRD-112. Des symptômes de toxicité ont limité l'usage de tous ces agents à des degrés variés. D'une manière générale on a été amené à conclure qu'il est nécessaire de disposer d'agents antifungiques plus actifs et moins toxiques pour que les effets thérapeutiques soient satisfaisants dans l'histoplasmose pulmonaire chronique. De nouveaux essais de ces agents dans la forme disséminée de l'histoplasmose pourraient être utiles.

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Serologic tests were performed by the U. S. Army Medical School Laboratory through the cooperation of Miss Charlotte Campbell, Mycologist.

REFERENCES

(1) Sutliff, W. D., Hughes, F., Ulrich, E., and Burkett, L. L.: Active chronic pulmonary histoplasmosis, A.M.A. Arch. Int. Med., 1953, *92*, 571.

(2) Loewen, D. F., Procknow, J. J., Loosli, C. G., and Tanzi, F.: Chronic active pulmonary histoplasmosis with cavitation; a clinical and laboratory study (to be published).

(3) Brasher, C. A., and Furcolow, M. L.: Chronic cavitary histoplasmosis as a problem in tuberculosis sanatoria, Tr. Fourteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1955, p. 288.

(4) Sutliff, W. D., and Campbell, C. C.: Serologic screen tests for the systemic mycoses, Tr. Fourteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1955, p. 285.

(5) Christie, A., Middleton, J. G., Peterson, J. C., and McVickar, D. L.: Treatment of disseminated histoplasmosis with ethyl vanillate, Pediatrics, 1951, *7*, 7; Am. J. Dis. Child., 1950, *80*, 874.

(6) Ludwig, K. A., Murray, F. J., Smith, J. K., Thompson, C. R., and Werner, H. W.: Laboratory studies on β-diethyl amino-ethyl fencholate, a new antifungal agent, Antibiot. and Chemother., 1954, *4*, 56.

(7) Brown, R., and Hazen, E. L.: Nystatin and actidione: Two antifungal agents produced by *Streptomyces noursei* in *Therapy of Fungus Diseases*, Little, Brown and Co., Boston, 1955, p. 164.

REVIEW OF CASES OF NONTUBERCULOUS DISEASE ORIGINALLY DIAGNOSED PULMONARY TUBERCULOSIS[1]

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INTRODUCTION

The rise of chest surgery has widened the knowledge of lung disease and awakened the physician to some of his past diagnostic mistakes. An example of this type of situation is presented by the patient who is treated for years for tuberculosis and then is found to have sarcoidosis after examination of a lung biopsy specimen. This newly gained knowledge along with the responsibility for administering chemotherapy to the proper patient has invited additional caution before diagnosing tuberculosis.

What are the most common conditions apt to be mistaken for tuberculosis? How often are such mistakes made and how can they be avoided? In an effort to answer some of these questions, cases of patients with nontuberculous disease admitted to the tuberculosis section of Fitzsimons Army Hospital for a three-year period were reviewed. All such patients were sent in with a diagnosis of tuberculosis or suspected tuberculosis.

During the period covered, 3,422 patients were admitted and 608 (17.8 per cent) of these patients were ultimately discharged with the diagnosis of nontuberculous disease (table 1). Each year the percentage of such cases was remarkably consistent. The classification of the various conditions encountered is presented in table 2. In table 3 the leading ten nontuberculous conditions observed during the three-year period are listed. They comprise 77 per cent of the total after excluding the patients in whom no pulmonary disease was found. The subsequent discussion will be confined to the most frequently encountered ten categories of nontuberculous disease; the case examples are drawn from the 1954 material (table 4). It will be noted that the leading conditions in 1954 are the same as are presented in table 3, but the order of rank is a little different.

OBSERVATIONS

Pulmonary Fibrosis

Pulmonary fibrosis accounts for 26 per cent of the first ten categories of nontuberculous pulmonary disease. Small areas of fibrosis, usually in the upper lung fields, are present which do not appear to be potentially significant. The tuberculin test can be positive or negative. (The typical apical fibrocalcified residua associated with tuberculosis are not included.) There are no symptoms or significant physical findings. In assigning cases to this arbitrary category the

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TABLE 1

NUMBER OF ADMISSIONS TO THE TUBERCULOSIS SECTION AND NUMBER OF NONTUBERCULOUS DISCHARGES
(1952-1953-1954)

	1952	1953	1954	Total
Total admissions	1,315	1,158	949	3,422
Total of nontuberculous discharges	239	207	162	608
Percentage of nontuberculous discharges	18	18	17	Average 17.8

most important single factor is the presence of unchanging densities in the roentgenogram. In a third of the patients the outcome of gastric cultures for tubercle bacilli is awaited before discharge. As refinements in diagnosis continue, the percentage left in this group will decline.

Case 1. A 36-year-old white female was admitted to the hospital in November, 1954, with a diagnosis of suspected tuberculosis. She had a history of pleurisy when sixteen years old, requiring strapping of the chest. No roentgenograms were obtained. In 1948 the patient had "virus pneumonia" with fever and dry cough, and rested for six weeks. In October, 1954, she had lower abdominal pain diagnosed as "parametritis." A routine chest film obtained at this time revealed bilateral apical infiltration. Prior to admission two sputum specimens were reported as positive for tubercle bacilli on microscopy, but it was remarked that the bacteria were "atypical." On admission to Fitzsimons Army Hospital, skin tests were: tuberculin, negative; coccidioidin, negative; and histoplasmin, positive. Chest films, including laminagrams, were interpreted as showing apical fibrosis with cystic changes (figure 1). Bronchoscopy and bronchograms were not remarkable. Diagnosis: fibrocystic disease, upper lobes; etiology undetermined.

This case illustrates a "false positive" sputum, which was encountered in 12 per cent of the whole series. Most of these were smears of sputum or gastric contents. All efforts at this hospital to recover the organisms from these patients failed. In the above case, the "atypical" organisms were not cultured.

Pneumonia

Pneumonia was encountered in 13 patients, 4 of whom had patches of pneumonia in upper lung fields which were slow in resolving. The cause for the slow resolution was not always evident; sometimes it was thought to be due to inadequate chemotherapy. In 2 patients, the pneumonitis occurred in emphysematous lungs. Two patients had intrapleural fluid with their pneumonia; in 3, the disease was classified as suppurative pneumonia; 2 of these patients gave an associated history of loss of consciousness.

Case 2. A 26-year-old white male soldier developed a headache in March, 1954. Subsequently a chest film indicated pneumonia. The patient was given antimicrobials for three days and quickly became asymptomatic; however, one sputum smear was reported as showing tubercle bacilli.

On admission to Fitzsimons (March 23, 1954), all skin tests were negative (tuberculin, histoplasmin, coccidioidin), and sputum cultures also were negative for tubercle bacilli.

Physical examination was normal. A chest roentgenogram showed a few linear infiltrates

TABLE 2

ANALYSIS OF PATIENTS WITH NONTUBERCULOUS DISEASE ADMITTED TO FITZSIMONS ARMY HOSPITAL WITH AN ORIGINAL DIAGNOSIS OF TUBERCULOSIS
(1952–1953–1954)

Discharge Diagnosis	1952	1953	1954	Three-Year Total
Infection and sequelae				
Fibrosis and/or calcification, pulmonary	40	36	27	103
Pulmonary mycotic and parasitic disease				
Coccidioidomycosis	28	10	7	45
Histoplasmosis	16	18	14	48
Other fungi	0	2	0	2
Schistosomiasis	0	0	1	1
Paragonimiasis	1	0	0	1
Bronchiectasis	24	16	11	51
Pneumonia	34	13	13	60
Pleurisy	7	6	13	26
Bronchitis, chronic	3	3	4	10
Lung abscess	3	1	6	10
Broncholithiasis	0	0	1	1
Lymphadenopathy, mediastinal	3	0	0	3
Emphysema, pulmonary	3	3	3	9
Empyema	0	2	1	3
Granuloma, etiology undetermined	0	11	7	18
Pneumoconiosis (silicosis)	3	3	0	6
Tumors and cysts				
Neoplasm, lung	4	10	8	22
Bronchogenic cyst	1	3	0	4
Cyst of lung	0	1	2	3
Allergic disorders	1	1	1	3
Congenital anomalies and structural changes				
Achalasia, esophagus	1	0	0	1
Pneumothorax, spontaneous	3	2	0	5
Undiagnosed lesion, probably due to agenesis	0	0	1	1
Trauma				
Hematoma, lung	5	1	0	6
Fibrosis of pleura	1	1	0	2
Fractured ribs with traumatic pneumonitis	0	1	0	1
Hemothorax, due to trauma	0	0	1	1
Foreign bodies, aspiration				
Lipoid pneumonia	1	1	0	2
Foreign body retained	1	0	0	1
Blood, vascular, and circulatory disorders				
Pulmonary embolism with infarction	4	0	1	5
Vascular anomaly, arteries, right upper lobe	1	0	0	1
Hemoptysis, cause undetermined	2	2	2	6
Disease of unknown cause				
Sarcoidosis	1	5	12	18
Xanthomatosis	0	1	0	1
Eosinophilic pneumonopathy	0	0	3	3
Artefacts, extrinsic shadows	0	0	0	0
Combined lesions				
Inactive tuberculosis with other pulmonary disease	7	0	0	7
Inactive tuberculosis with extrapulmonary nontuberculous disease	4	7	0	11
Unclassified and other lesions				
Observation, medical, pulmonary disease, none found	31	20	8	59
No pulmonary disease, but extrapulmonary disease	6	15	10	31
Undiagnosed lesions	0	12	5	17
Totals	239	207	162	608

TABLE 3

LEADING NONTUBERCULOUS CONDITIONS ENCOUNTERED, THREE-YEAR TOTAL (1952–1953–1954)

	Number	Per Cent
Fibrosis, pulmonary	103	25.6
Pneumonia	60	15.0
Bronchiectasis	51	12.7
Histoplasmosis	48	12.0
Coccidioidomycosis	45	11.2
Pleurisy	26	6.5
Neoplasm (malignant)	22	5.4
Sarcoidosis	18	4.4
Granuloma (etiology undetermined)	18	4.4
Lung abscess	10	2.4
	401	100
Top ten nontuberculous conditions	401	66
No pulmonary disease found	90	15
Remaining miscellaneous conditions	117	19
Total	608	100

TABLE 4

LEADING NONTUBERCULOUS CONDITIONS ENCOUNTERED, 1954

Fibrosis, pulmonary	27
Histoplasmosis	14
Pleurisy	11
Pneumonia	13
Sarcoidosis	12
Bronchiectasis	13
Neoplasm	7
Granuloma, etiology undetermined	7
Coccidioidomycosis	7
Lung abscess	6
Total	162

in an apicoposterior segment of the left upper lobe, with the remainder of the lung fields clear. Subsequent films showed complete clearing in one month. A review of the initial film (March 8, 1954) showed pneumonic consolidation in the left upper lobe, with a suggestion of cavitation which cleared markedly in a few days (figure 2*A*, *B*, *C*).

In this instance, a review of the initial films virtually clenched the diagnosis. The one smear positive for tubercle bacilli was disturbing, but the negative tuberculin skin test obviated further concern.

Bronchiectasis

The 11 cases of bronchiectasis did not follow any pattern. Three patients had upper lobe involvement only, while the remainder had two or more lobes af-

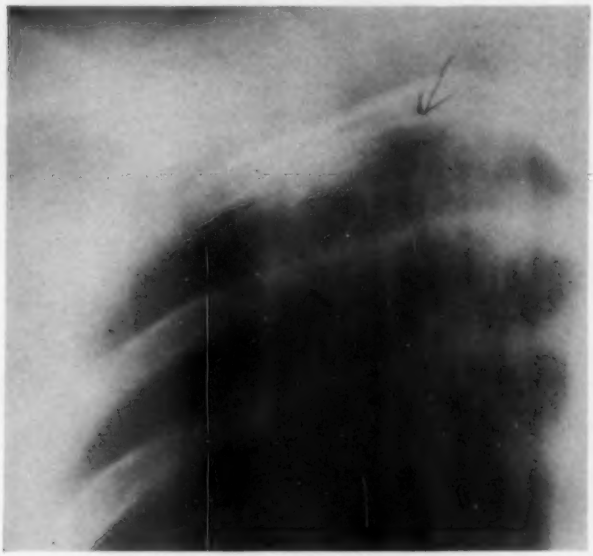

FIG. 1. *Case 1*—Fibrosis. Laminagram showing fibrocystic changes, right upper lobe.

fected. In 5 the roentgenographic appearance suggested tuberculosis. Two were referred to the hospital because of "false positive" smears and one primarily because of hemoptysis.

Case 3. A 24-year-old white male soldier was admitted to the hospital in April, 1953, from Tokyo General Hospital with a diagnosis of active tuberculosis. He had recently developed a dry cough and malaise in Korea where his chest film was found to be abnormal. On admission, inspiratory and expiratory rales were heard over the left apex. The skin tests were: tuberculin, positive; coccidioidin and histoplasmin, negative. Ova of hookworm were found in stools. All sputum studies were negative for tubercle bacilli. A chest film showed fibrosis in both upper lung fields, with a paucity of markings in the left lung field (figure 3). The patient was started on isoniazid-streptomycin, but no roentgenographic improvement was noted. A bronchogram revealed bronchiectasis involving segments of the left lower lobe with absence of filling of the left upper lobe (figure 5). Bronchospirometric studies showed 89 per cent oxygen consumption on the right and 11 per cent on the left. The maximal breathing capacity was 85 liters per minute, or 72 per cent of predicted. A one-second vital capacity was 1.9 liters, or 61 per cent of predicted. An angiocardiogram showed almost complete lack of filling of the left pulmonary artery (figure 4). The patient's symptoms continued to be minimal so a left pneumonectomy which had been considered was not deemed indicated.

Because of the upper lobe roentgenographic changes suggestive of tuberculosis and the absence of cough, bronchiectasis was not suspected despite the extensive changes present. The patient had persistent rales, an important indication in the diagnosis of bronchiectasis. The persistence of rales in patients receiving antituberculous drugs does not usually indicate the presence of uncomplicated tuberculosis.

Case 2—Pneumonia

FIG. 2A. Roentgenogram showing infiltration in left upper lobe. *B*. Roentgenogram obtained a week later showing moderate clearing. *C*. Roentgenogram obtained a month after figure *B* showing complete clearing.

Case 3—Bronchiectasis

FIG. 3. Roentgenogram showing linear and nodular densities in upper lobes.

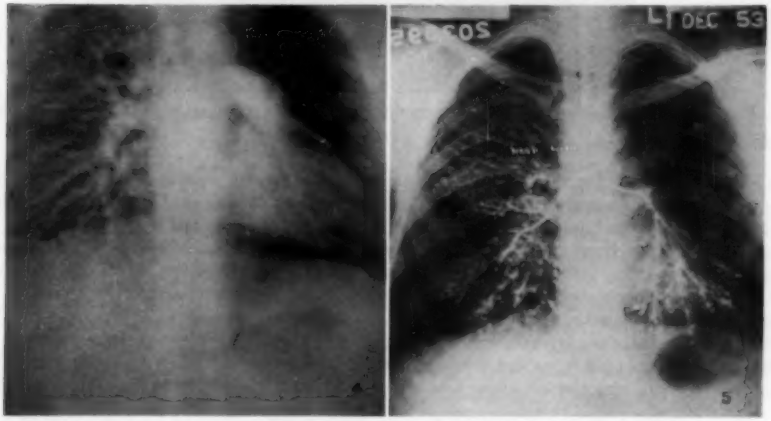

FIG. 4. Angiogram showing absence of filling of left pulmonary artery.

FIG. 5. Bronchogram showing bronchiectasis in left lower lobe, with lack of filling of upper lobe.

Histoplasmosis

Histoplasmosis was found in 13 patients. Four lesions appeared as "coin" lesions and were resected for diagnosis. The diagnosis in one patient was made because of a sputum culture which was positive for *H. capsulatum* This case is described below. In the remaining 8 patients the diagnosis was based on a combination of a positive complement-fixation test plus suggestive roentgenographic changes.

Case 4. A 58-year-old male was admitted to the hospital in February, 1954, with a history of fatigue and with occasional bouts of ankle edema and shortness of breath for several months. The patient had a slight productive cough with grayish sputum. There had been

FIG. 6. *Case 4*—Histoplasmosis. Roentgenogram showing infiltration with cavitation, left upper lobe.

a history of cough for thirty years associated with cigarette smoking. He lived in Ohio where chest roentgenograms were made a month prior to admission. Physical examination of the chest revealed post-cough rales and a few wheezes over the left upper lung field. The remainder of the examination was not remarkable. The skin tests were: tuberculin, positive; histoplasmin, 1:100 and 1:10, negative; coccidioidin, negative. Serologic tests for fungal infections showed a positive complement fixation for histoplasmosis, with a titer of 1:512. Repeated sputum examinations and cultures were negative for tubercle bacilli. Bronchoscopy was negative; however, cultures of bronchial washings produced growth of *Histoplasma capsulatum*, as did several sputum cultures. A chest film revealed a mottled infiltration involving the left upper lobe with several cavities 1–2 cm. in diameter (figure 6).

This man's chest roentgenogram revealed a condition that looked exactly like advanced tuberculosis, but symptoms were milder than would be expected. The negative histoplasmin skin test is unusual. In the experience of the writers, this test is nearly as dependable as the tuberculin test.

Coccidioidomycosis

The incidence of coccidioidomycosis in military personnel is relatively high because of the many soldiers stationed in endemic areas. When the skin test is positive, a history of residence in such an area is almost always obtained. In the 8 cases encountered, the diagnosis was established by sputum culture in 3 and by surgery in 5 patients. All had upper lobe disease.

Case 5. A 41-year-old white male airman was admitted to the hospital in December, 1953, with a diagnosis of minimal pulmonary tuberculosis and diabetes. The onset was in August,

FIG. 7. *Case 5*—Coccidioidomycosis. Roentgenogram showing a small round infiltrate, right first intercostal space.

1953, with fever and right upper chest pain which was aggravated by breathing. The patient was hospitalized at Lackland Air Force Base Hospital where a chest film revealed a lesion in the right upper lobe. He was treated for pneumonia, but the lung infiltrate did not respond, so he was started on streptomycin and PAS therapy and transferred to Fitzsimons.

On admission to this hospital skin tests were: tuberculin, positive; coccidioidin, positive; histoplasmin, negative. Sputum cultures were negative for tubercle bacilli. Serologic tests for fungi were negative. Aspergillus was cultured from one specimen. Urine showed an occasional trace of sugar but fasting blood sugar was 100 mg. per 100 ml. A chest film revealed a nodular infiltrate projected through the right first intercostal space anteriorly with a few lesser adjacent densities (figure 7). Chemotherapy for tuberculosis was continued, isoniazid being substituted for PAS. Diabetes was controlled by diet. No change in the lesion was noted after five months of drug therapy so an exploratory thoracotomy was performed and wedge resection of the round lesion in the apical segment was performed. The pathologic diagnosis was coccidioidomycosis, positive cultures for *Coccidioides immitis* being obtained.

This is a typical case, starting with a patch of pneumonitis which subsided but did not entirely clear, leaving a nodular residual indistinguishable from tuberculosis. The failure of the lesion to change after months of antituberculous drugs finally resulted in the decision to explore.

In the presence of coccidioidomycosis, the coccidioidin skin test is negative in about 30 per cent of the patients, so it is important to do a complement-fixation test whenever suspicions are aroused.

Pleurisy

In 1954, 100 patients having pleurisy with effusion were admitted to the tuberculosis service, 13 of whom were considered to have nontuberculous dis-

FIG. 8. *Case 6*—Pleurisy with effusion. Roentgenogram showing a small effusion, right base.

ease. Four had negative tuberculin skin tests; 4 had diagnoses made only after exploration and decortication; 2 had disease which could not be explained. In those on whom surgery was performed the pathologic diagnosis was simply pleurisy, chronic; etiology, undetermined.

Case 6. A 21-year-old white male soldier was admitted to Fitzsimons in October, 1953, with a diagnosis of serous acute pleurisy, presumably tuberculous, and syphilis, late latent seropositive only.

The patient gave a history of sudden onset, in August, 1953, of chest pain aggravated by breathing and associated with a 10 pound weight loss. No chills, fever, cough, or sputum were noted. A chest roentgenogram revealed the presence of fluid in the right base (figure 8).

On admission skin tests (tuberculin, histoplasmin, and coccidioidin) were negative. Sputum and pleural fluid cultures were negative for tubercle bacilli. The hemogram was within normal limits. A serologic test for syphilis was positive. A right axillary node biopsy revealed no abnormality. Bronchoscopy was negative. A bronchogram indicated poor filling of right lateral and posterior basal segments. An exploratory thoracotomy was performed and a pleural "peel" was removed with infolded areas of lung. All lobes and interlobar fissures were freed. No evidence of parenchymal lesions was found. The pathologic diagnosis was chronic nonspecific pleuritis. The persistence of fluid was attributed to trapping of basal segments, the resultant space filling with fluid.

Deciding whether or not an effusion is tuberculous in origin is often difficult and places a great responsibility on the physician. In this case the negative tuberculin test gave the answer. If the effusion is tuberculous, long-term drug therapy will control the disease and prevent reactivation, while without it the relapse rate is very high. This suggests that it is better to overdiagnose tuberculosis; yet unnecessary treatment for the disease for a year is hard to defend. All of which means that exploratory thoracotomy finds justification when reasonable doubt exists (1).

Neoplasm

Of the 7 neoplasms encountered in 1954, 3 were epidermoid carcinoma, 2 bronchiolar carcinoma, one bronchial adenoma, and one pulmonary metastasis from adenocarcinoma of the stomach. Four patients were referred to the hospital because the roentgenographic appearance suggested tuberculosis; 2, because of a "false positive" sputum (resected specimens revealed no tuberculosis), and one because of unexplained cough that proved to be due to a bronchial adenoma.

Case 7. A 28-year-old white male was admitted to Fitzsimons Hospital in September, 1953. The history dated back to 1946 when he had pneumonia following an appendectomy and then two weeks later a right spontaneous pneumothorax. It was thought that the collapse was due to rupture of a bleb, but tuberculosis was also considered. For the next four years the patient had no symptoms of chest disease. Following his induction into the Army in 1950 he began to have frequent colds with productive cough. A chest film taken in 1952 was said to be normal; however, on his separation from the Service in July, 1953, the roentgenogram was abnormal. Cystic changes and an irregular area of infiltration were present in the right upper lobe (figure 9). One of seven sputum cultures obtained prior to admission was reported positive for tubercle bacilli.

On admission the patient was asymptomatic. In view of the one sputum positive for *M. tuberculosis*, antituberculous drugs were started, but tumor was considered because of progressive enlargement of the infiltration since July, 1953. Internal spirometric studies revealed that the right lung consumed only 23 per cent of the oxygen.

An exploratory thoracotomy was performed and an extensive bullous emphysema involving all lobes was encountered. An infiltrative lesion was found in the right upper lobe which frozen section revealed to be a moderately anaplastic large-cell bronchiolar carcinoma. For this reason, pneumonectomy was performed. There was no evidence of hilar metastasis. Of additional interest were pulmonary histoplasmosis and microscopic abscesses due to moni-

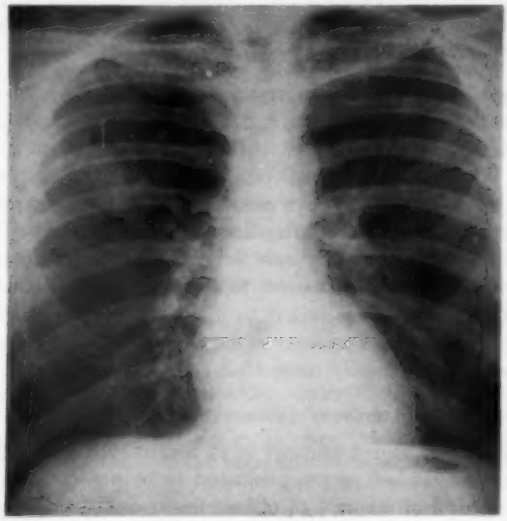

FIG. 9. *Case 7*—Bronchiolar carcinoma. Roentgenogram showing a patch of infiltration seen in the right upper lobe with adjacent emphysema.

liasis. Tuberculosis was not confirmed histologically. Metastases to the cervical lymph nodes developed six months postoperatively.

There are no specific diagnostic aids that especially commend themselves for the early recognition of neoplastic disease, short of a high index of suspicion. All of the patients in this category came to surgery, but only one survives at the present writing.

Sarcoidosis

Of the 12 patients with pulmonary sarcoidosis, 6 showed a diffuse symmetrical linear and nodular infiltration. Two had only hilar adenopathy. In several of this series, sarcoid changes were found in biopsy material from more than one site: liver, 6; axillary node, 4; lung, 3; scalene fat pad, 3. The tuberculin test was positive in 6 patients.

Case 8. A 26-year-old white male, a repatriated prisoner of war, had been captured by Communist forces in February, 1951, following which he had cough and fever for two months. While a prisoner, he also had frequent episodes of dysentery and intermittent night blindness. A 20 pound weight loss was also noted. Roentgenograms obtained upon his liberation in August, 1953, were reported to be abnormal.

On admission to Fitzsimons in November, 1953, the patient was essentially asymptomatic. Parasites and ova were found in stools, and appropriate treatment was given. The physical examination was not remarkable. The skin tests were: tuberculin, positive; histoplasmin, positive; coccidioidin, negative. A serologic test for fungi was negative. Gastric cultures were negative for tubercle bacilli. A chest film revealed small ill-defined densities in both upper lung fields (figure 10).

A review of previous chest films from 1945 to 1950 showed no abnormalities. Laminagrams revealed fine fibrous-appearing nodular areas throughout the subapical regions, giving a reticulated pattern. Because a nontuberculous process was suspected, a thoracotomy was done. Tissue examination revealed a chronic noncaseating granulomatous pneumonitis consistent with pulmonary sarcoidosis of the nodular type.

The above case illustrates the importance of biopsy in the presence of nodular and linear fibrosis which does not quite fit the usual tuberculosis pattern. Without it this patient might have been subjected to long-term antituberculous chemotherapy. When pulmonary sarcoidosis is suspected, exploration of the supraclavicular space is now always made first. This procedure yields tissue from which a diagnosis can be made in a high percentage of cases (2).

Sarcoidosis is one of the most frequent causes of bilateral hilar adenopathy, while tuberculosis infrequently causes bilateral enlargement. Another point to consider in hilar adenopathy is not to use X-ray therapy before tissue examination has established the presence of neoplastic disease. Regression of hilar enlargement does not necessarily mean that a tumor is present.

Granuloma of Undetermined Etiology

Granuloma of undetermined etiology is an arbitrary designation for patients in whom granulomatous lesions are presumed to be present. There were 7 patients so designated, 4 of whom had diffuse linear and nodular changes, and 3 had localized nodules. Biopsy was recommended in 5 but performed only in 3

FIG. 10. *Case 8*—Sarcoidosis. Apical view showing fine nodular and linear densities, right upper lobe.

patients. The latter had exploratory thoracotomies, but tissue examination did not yield a specific diagnosis. It is well to emphasize that the pathologist not infrequently encounters changes which must simply be termed a nonspecific granulomatous reaction. The use of special bacteriologic staining techniques for fungi helps to reduce the number of cases so designated.

Lung Abscess

Of the six abscesses, two followed electro-shock therapy; two were in debilitated prisoners of war; one occurred after a bout of drunkenness; and in one, the cause was undetermined. All of the lesions were in the upper lobe (four right, two left) with two requiring resection.

Case 9. A 23-year-old white male soldier with four years of active duty, including two years in prison camp, was admitted to Fitzsimons in August, 1953. The first symptoms resulted from a bombshell blast in Korea in September, 1951, following which he was captured by the Chinese and placed in a prison hospital for three weeks. In retrospect, he remembers a small laceration over the right cheek and moderate bleeding from the nose and mouth for three days. For a month he was unable to speak clearly and swallowed with considerable difficulty (a roentgenogram obtained two years later in Japan revealed an 8 mm. metallic foreign body in the region of the left tonsillar fossa). After leaving the prison hospital he had repeated respiratory infections. In April, 1953, the patient developed night sweats, malaise, and productive cough, raising yellow sputum which was not foul. In June, 1953, he

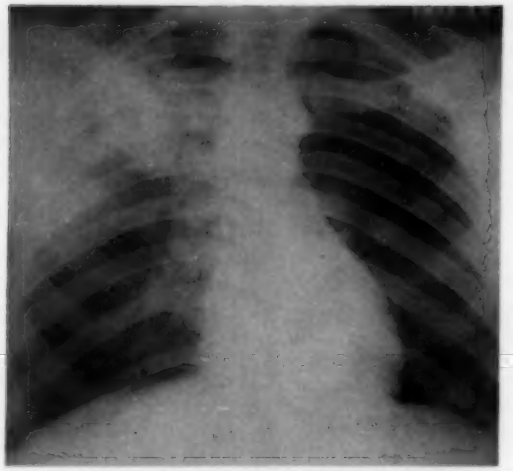

FIG. 11. *Case 9*—Lung abscess. Roentgenogram showing diffuse infiltration, right upper lung field.

was given a short course of penicillin, which resulted in brief symptomatic improvement. On several occasions he coughed blood. During imprisonment he lost 40 pounds.

Upon admission, examination of the lungs revealed impairment to percussion high in the right axilla, with increase in breath sounds over the right apex. Clubbing of the fingers was noted. There was permanent incomplete weakness of the left side of the tongue due to injury to the hypoglossal nerve by a shell fragment. A chest film showed parenchymal infiltration involving the entire right upper lobe (figure 11). Bronchoscopy revealed purulent secretions from the right upper lobe but no endobronchial disease. A thoracotomy was done and the right upper lobe was removed. The pathologic diagnosis was chronic pulmonary abscess with bronchiectasis and pleuritis; etiology, undetermined.

In the presence of abscess it is rare not to elicit a history of aspiration. The patient's difficulty in swallowing was a factor in the above case.

General Comment

In 1932, one of the present writers made a study similar to the present one, based on experience in a large sanatorium (4). Since that time there have been added to the top ten diseases in the category: histoplasmosis, sarcoidosis, and neoplasms. Coccidioidomycosis is also new to the list but is only a regional diagnostic problem. Unexplained hemoptysis in the patient more than forty years old now first evokes suspicion of neoplastic disease rather than tuberculosis (5). Upper lobe bronchiectasis is still apt to be confused with tuberculosis, but lower lobe bronchiectasis is now seldom misdiagnosed.

Today physicians are less sure of the significance of lung shadows, especially in the upper lung fields, and feel an increasing need for clarification which is presently met by skin tests, serologic tests, detailed roentgenographic studies and—most important—biopsy.

The present study prompts the emphasis of a few points:

All previous chest roentgenograms of the patient should be immediately obtained and carefully reviewed. Many patients have had at least a small chest film at some time in their lives. Evidence of a stable lesion, as determined on serial films, was the deciding factor in eliminating the possibility of active tuberculosis in at least 30 per cent of the patients in the present study.

A single postero-anterior film of the chest is inadequate. An apical view should always be obtained whenever there is the least question of upper lobe disease. Oblique views are next in importance and then lateral views. Laminagrams are invaluable and constitute the greatest single diagnostic advance of recent years in chest disease.

At least three skin tests should be applied (tuberculin, histoplasmin, and coccidioidin). For tuberculin testing it is customary initially to apply first-strength PPD and, if the patient's reaction is negative, then intermediate or second-strength PPD. (About 10 per cent of patients with active tuberculosis fail to respond to first-strength PPD. For routine testing or hospital personnel, intermediate PPD is used.) Histoplasmin and coccidioidin tests are given in 1:100 dilution and are read at twenty-four and forty-eight hours. The tuberculin reaction is read to the best advantage at seventy-two hours.

Of the 162 patients in the 1954 series the percentage of positive reactors to the three skin tests were: tuberculin, 60 per cent; histoplasmin, 34 per cent; and coccidioidin, 15 per cent.

A minimum of three sputum specimens or gastric aspirates for culture for tubercle bacilli and fungi should be obtained. A cytologic study is done when suspicion of neoplastic disease exists. The diagnosis of tuberculosis should not be based on a single smear positive for tubercle bacilli. When atypical organisms are noted, guinea pig inoculation, as well as culture, is indicated.

In 1954, 12 per cent of the series had "false positive" sputum prior to admission. At this hospital, in the presence of active tuberculosis, tubercle bacilli are found in about 70 per cent of the patients. This means that in many patients negative bacteriology does not exclude active disease.

The history of places of past residence should be carefully elicited, even though the fungal skin tests are negative. The occupational history should not be overlooked, and careful questioning is necessary with respect to loss of consciousness, regurgitation, alcoholism, and trauma. A detailed account of the antimicrobials received is also very important.

Auscultation of the chest is important. The presence of moisture can only be detected by the stethoscope. Its persistence means abnormality and often points to the need for bronchoscopy and bronchography when the roentgenograms are clear. It must be kept in mind that the presence of expiratory wheezes does not necessarily indicate the presence of asthma.

The availability of a reasonably safe water-soluble contrast media has invited the freer use of bronchography. With it a better picture is obtained and the lung fields are not subsequently obscured by retained oil. A careful mapping of the bronchial tree not only reveals areas of bronchiectasis, but various abnormal

bronchial patterns associated with pulmonary fibrosis or emphysema. Bronchoscopy should be done before bronchography.

Therapeutic drug trials should not be depended upon to make the diagnosis. If routine procedures are not rewarding, a tissue examination without delay is usually best. Staring at roentgenograms and idly speculating cannot be justified. The advent of specific chemotherapy has made it increasingly important to decide whether or not tuberculosis is present. In this series, 30 per cent of the patients had biopsies. If diffuse pulmonary disease is suspected, exploration of the supraclavicular space is usually made first. When it is necessary to examine the lungs, an open thoracotomy is presently preferred to simple lung or pleural biopsy (6, 7).

Summary

Over a three-year period, 608 (18 per cent) of the patients admitted to the tuberculosis section of Fitzsimons Army Hospital were found not to have tuberculosis. Ten conditions comprised 77 per cent of the total lung disorders encountered. The great importance of the tuberculin test and sputum culture is emphasized. In the 1954 series, 40 per cent of the patients were tuberculin negative and 12 per cent had "false positive" sputum reports. The need for prompt biopsy of material in obscure lung lesions is stressed.

Sumario

Repaso de Casos No Tuberculosos con un Diagnóstico Inicial de Tuberculosis Pulmonar

Durante un período de tres años, en 608 (18 por ciento) de los enfermos recibidos en la sección de tuberculosis del Hospital Militar Fitzsimons se descubrió que no tenían tuberculosis. Diez dolencias formaban 77 por ciento del total de trastornos pulmonares encontrados. Recálcase la gran importancia de la prueba de la tuberculina y del cultivo del esputo. En la serie de 1954, 40 por ciento eran negativos a la tuberculina y 12 por ciento tenían informes "seudopositivos" para el esputo. Acéntuase la necesidad de una biopsia pronta del tejido en las lesiones obscuras.

Resume

Examen de cas non tuberculeux pour lesquels un diagnostic original de tuberculose pulmonaire avait été porté

Au cours d'une période de trois années, il a été découvert que 608 (18%) des malades admis dans le service de la tuberculose de l'hôpital Fitzsimons, n'étaient pas tuberculeux. On relevait dix différents types d'affections dans 77% des cas pour la totalité des lésions pulmonaires observées. Les auteurs insistent sur l'importance du test à la tuberculine et de la culture de l'expectoration. Dans les séries de 1954, 40% des cas avaient des réactions négatives à la tuberculine et dans 12% cas l'expectoration donnait des résultats "faussement positifs". La nécessité de pratiquer des prélèvements dans les lésions mal définies est mise en évidence.

REFERENCES

(1) Stead, W. W., Eichenholz, A., and Strauss, H.-K.: Operative and pathologic findings in twenty-four patients with effusion, presumably tuberculous, Am. Rev. Tubere., 1955, *71*, 473.

(2) Shefts, L. M., Terrill, A. A., and Swindle, H.: Scalene node biopsy, Am. Rev. Tubere., 1953, *68*, 591.
(3) Hodgson, C. H., Olsen, A. H., and Good, C. A.: Bilateral hilar adenopathy: Its significance and management, Ann. Int. Med., 1955, *43*, 83.
(4) Burke, R. M.: Review of non-tuberculous cases originally diagnosed as pulmonary tuberculosis, Minn. Med., 1932, *15*, 18.
(5) Moersch, H. J.: Clinical significance of hemoptysis, J. A. M. A., 1952, *148*, 1461.
(6) Effler, D. B., Van Ordstrand, H. S., McCormack, L. J., and Gancedo, H. A.: Lung biopsy, Am. Rev. Tubere., 1955, *71*, 668.
(7) Theodos, P. A., Allbritten, F. F., Jr., and Beckenridge, R. L.: Lung biopsy in diffuse pulmonary disease, Dis. of Chest, 1955, *27*, 637.

EVALUATION OF CLINICAL AIDS TO THE DIAGNOSIS OF CHRONIC PROGRESSIVE CAVITARY HISTOPLASMOSIS[1]

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Introduction

Pulmonary cavitation due to histoplasmosis was first described in 1948 (1, 2) as an unusual form of the disease. Sutliff and associates (3), in 1953, collected seven cases from the literature and described in detail the clinical features of four additional cases. The striking resemblance of this form of histoplasmosis to that of cavitary tuberculosis has led to a careful search for this entity among the patients in a tuberculosis sanatorium located in the "high area" of histoplasmin skin test sensitivity. Using serologic tests for histoplasmosis as a screening procedure, a large number of cases have been uncovered. Furcolow and Brasher (4) recently reported nineteen mycologically proved cases of chronic progressive cavitary histoplasmosis uncovered by this study and speculated as to the general prevalence of this disease in tuberculosis sanatoriums. The increasing number of reported cases and the problems encountered in distinguishing cavitary histoplasmosis from cavitary tuberculosis has led to the present report. It is the purpose of this paper to present the clinician with available information on the usefulness and limitation of the tools available in establishing the diagnosis of chronic progressive cavitary histoplasmosis.

Materials and Methods

The present study began during the spring of 1954 when a serologic survey for histoplasmosis was undertaken among the patients of the Missouri State Sanatorium. This sanatorium has an average daily census of 550 patients, 85 per cent of whom are white. Following the initial survey, the study has been continued by skin testing with tuberculin and histoplasmin and serologic screening for histoplasmosis of every admission.

Intradermal skin tests were administered and read by the ward physician in each case. Purified protein derivative (PPD) in a dosage of 0.0001 mg. was employed in the tuberculin skin tests. The histoplasmin used was 0.1 ml. of lot HKC-5 diluted 2 parts to 1,000, which is equivalent to other standard histoplasmins. All skin tests were read in forty-eight hours, and any reaction of 5 mm. or more of induration was considered positive.

The sera of all patients were studied for complement-fixing antibodies, using histoplasmin as the antigen. These studies were done by Drs. Joseph Schubert and Kenneth Walls at the Communicable Disease Center, U. S. Public Health Service, Chamblee, Georgia. Approximately half the sera were also tested by complement-fixation tests with yeast phase antigen and a precipitin test with a purified histoplasmin antigen. The latter tests were performed by Dr. Samuel Salvin at the Rocky Mountain Laboratory, U. S. Public Health Service, Hamilton, Montana. Complement-fixation tests in a serum dilution of 1:8 or greater

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were considered positive. Precipitin tests in a dilution of 1:40 or higher were interpreted as positive.

Sputum cultures for fungi were performed on approximately 50 per cent of the patients with serologic evidence of histoplasmosis and in some others in whom the diagnosis of tuberculosis could not be established. Mycologic studies were performed both in the laboratory of the Missouri State Sanatorium and at the Kansas City Field Station Mycology Laboratory. Specimens sent to the Kansas City laboratory were mailed in sterile screw-capped sputum bottles to which penicillin and streptomycin (10,000 units per ml.) were added. Bacteriologic studies for tuberculosis, including smears, cultures and, in selected instances, guinea pig inoculations, were performed by the laboratory at the Missouri State Sanatorium.

Pathologic specimens obtained at the time of thoracic surgery on all of those patients with serologic evidence of histoplasmosis were stained with hematoxylin and eosin, as well as with the periodic acid Schiff and acid-fast stains. All sections were examined by Drs. David Gorelick and Henry Sweany of the Missouri State Sanatorium for typical intracellular yeasts of *Histoplasma capsulatum* and for acid-fast organisms.

Results

During the two years covered by this study, 1,800 sera were examined for antibodies against *H. capsulatum*. As a result, some 130 patients with positive serologic tests for histoplasmosis were found. These cases are called "serologically proved" cases. Mycologic studies on these cases have confirmed, in 33 instances, the diagnosis of cavitary histoplasmosis. These are called "mycologically proved" cases. The results of skin, serologic, and mycologic tests on 10 additional mycologically proved cases from another study will be included to lend greater statistical significance in the evaluation of these aids to the diagnosis of cavitary histoplasmosis.

Mycologically Proved Cases

Clinical manifestations: The clinical histories among the 43 proved cases of chronic progressive cavitary histoplasmosis uncovered by this study were found to be similar to those of cavitary tuberculosis, with which it is so often confused. These patients ranged in age from twenty-three to seventy-four years, with a mean age of fifty-five. Approximately 90 per cent of these patients were males, and all but one were white. The majority dated the onset of their illness several years prior to the present hospitalization and many have been hospitalized repeatedly with the diagnosis of "far advanced tuberculosis." Symptomatology was largely respiratory. Cough, productive of moderate amounts of mucopurulent sputum, occasionally blood streaked, was the major symptom. Bouts of low-grade fever, night sweats, weight loss, and fatigue were likewise common complaints. In general, the disease process was characterized by widely separated remissions and exacerbations. Progression of the disease, as evidenced by roentgenographic changes, was considered to have occurred in 11 of the 43 cases while under observation. Four deaths have occurred in this group. For detailed case descriptions, the reader is referred to the descriptions by Sutliff and co-workers (3) and others (5–12).

Roentgenographic findings: The roentgenographic findings in cavitary histoplasmosis were found to be indistinguishable from those of "cavitary" tubercu-

losis. The majority of the pulmonary lesions were considered to represent fibrocavitary disease in roentgenographic appearance. Early films revealed apical or subapical infiltrations which eventually progressed to single or multiple thick-walled cavities surrounded by considerable parenchymal disease. After a period of months, spread frequently occurred. This often appeared within the same lung, but spread to the opposite lung was not uncommon. Spread to the contralateral lung was frequently subapical in localization.

In many instances single or multiple calcified parenchymal lesions were found. Hilar adenopathy was infrequent, but calcified hilar nodes were noted quite frequently. The occurrence of active apical disease along with calcified lesions in tuberculin-negative patients suggests that this may well be a "reinfection" type of histoplasmosis. After multiple episodes of spread, the major portion of a lung may be "destroyed" and reduced to a contracted fibrotic mass of cavities. Of the 18 cases of cavitary histoplasmosis reported in the literature and the 43 mycologically proved cases presented in this report, the major involvement was confined to the upper lobes in all but 2 cases.

One patient was suspected of having a lung abscess which was confined to the right lower lobe (6). In the other instance, among the present series, saccular bronchiectasis of the lingula was found, probably resulting from the complications of a "middle lobe syndrome." Of the remaining, bilateral upper lobe disease was found in 26, the majority with multiple thick-walled cavities bilaterally. Unilateral lesions were found in the 33 remaining patients; 21 were confined to the right upper lobe and 12 to the left upper lobe.

Skin tests: Results of the histoplasmin and tuberculin skin tests on the 43 proved cases are presented in table 1. It should be noted that sensitivity to standard doses of histoplasmin was demonstrable in only 74 per cent of these mycologically proved cases. Fifty-one per cent were found to be positive reactors to intermediate strength tuberculin.

Serologic results: Serologic tests for histoplasmosis were performed on the sera of a majority of the proved cases on at least three occasions. In many instances ten or more determinations were performed during the course of the study. With the exception of several cases following surgical removal of the lesion and in one instance of experimental drug therapy, the serologic tests, once positive, remained consistently positive (13). Serum titers from 24 of the mycologically proved cases in which both the histoplasmin and yeast phase antigen complement-fixation tests were determined are presented in table 2. It should be noted

TABLE 1
RESULTS OF SKIN TESTS ON CULTURALLY PROVED CASES

	Histoplasmin	Tuberculin
Positive	31	20
Negative	10	19
Not done	2	3
Per cent positive	74	51

TABLE 2
COMPARISON OF THE COMPLEMENT-FIXATION TITERS OBTAINED WITH TWO ANTIGENS ON THE SERA OF 24 MYCOLOGICALLY PROVED CASES

Titer	Number of Patients	
	Histoplasmin Antigen	Yeast Phase Antigen
0	6	4
1:4	0	3
1:8	5	2
1:16	2	5
1:32	3	9
1:64 or more	8	1
Total positive (≥1:8)	18	17

TABLE 3
RESULTS OF COMPLEMENT-FIXATION TESTS EMPLOYING HISTOPLASMIN AS ANTIGEN ON THE SERA OF 19 MYCOLOGICALLY PROVED CASES

Titer	Histoplasmin Antigen
0	4
1:4	0
1:8	3
1:16	2
1:32	3
1:64 or more	7
Total positive (≥1:8)	15

that, of this group, 7 of the sera were negative (less than 1:8) to yeast phase antigen and 6 were negative to the histoplasmin antigen. However, in only 2 instances were both these tests negative. In one of these, a doubtful yeast phase titer of 1:4 and a positive precipitin test were found. In 11 of the cases positive titers were demonstrated to both of these antigens.

In general, higher complement-fixing titers were found with histoplasmin as the antigen. The highest titer among this group of patients using histoplasmin antigen was 1:512, while a titer exceeding 1:32 with the yeast phase antigen was found in only one instance. Employing both of these tests, circulating antibodies were demonstrated in 90 per cent of the mycologically proved cases. Precipitating antibodies in a titer of 1:40 or greater were demonstrated in approximately 60 per cent of these cases. In table 3 are presented the serologic results in 19 additional proved cases in which only the histoplasmin complement-fixation test was performed. Although tests using this antigen have been performed repeatedly during the period of observation, antibodies could not be demonstrated in more than 80 per cent of the culturally proved cases.

Mycology and bacteriology: In 39 of the 43 proved cases in this study the diagnosis was established by culturing *H. capsulatum* from the sputum. In the remaining 4 cases, the diagnosis was established both by pathologic demonstration of the fungus in tissue and by cultures of resected pulmonary tissue. Sutliff and

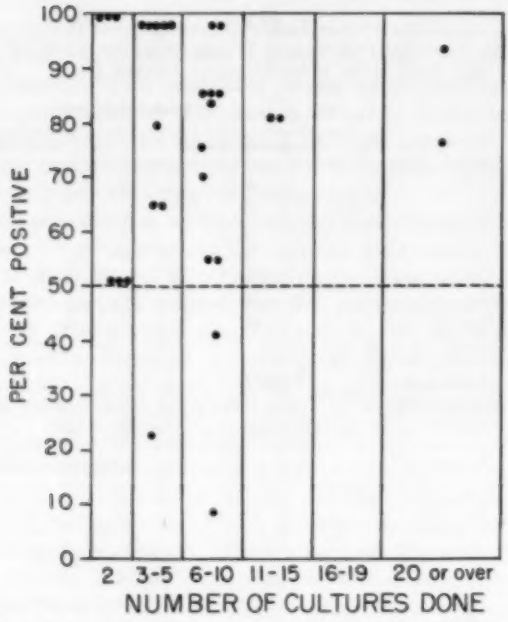

FIG. 1. Results of repeated sputum cultures for *Histoplasma capsulatum* among 31 mycologically proved cases.

associates (3) and Furcolow and Brasher (4) have stated that sputum cultures for *H. capsulatum* in the chronic cavitary form of the disease are frequently and repeatedly positive.

As a part of the present study, frequent specimens of sputum and gastric washings were cultured from 31 patients during the period of observation to establish the frequency with which positive cultures occur. In figure 1 are shown the number of cultures done on each of these patients and the per cent positive. With 3 exceptions, 50 per cent or more of the cultures done on an individual patient were positive for *H. capsulatum*. All 3 patients with less than 50 per cent positive cultures had a nonproductive cough and cultures of gastric washings had to be employed. A total of 212 cultures was performed on the entire group, of which 165 (77 per cent) were positive.

Patients with two or more positive cultures were followed with frequent cultures for periods of one to twenty-four months. Although the majority of these patients have received experimental drug therapy (MRD-112, 2-hydroxy-stilbamidine, aminostilbamidine, or nystatin), the cultures remain consistently positive for *H. capsulatum*. In only one instance was "sterilization" of the sputum accomplished. This patient received a sixty-day course of intravenous MRD-112[2] (β-diethylamino-ethyl fencolate), followed by a three-month trial of amebicide

[2] Wm. S. Merrell Co., Cincinnati, Ohio.

CT 686[4] (1,2 bis p (n-hexyl methylaminomethyl) phenoxy ethane dihydrochloride). Prior to administration of the amebicide, seventeen of twenty-three sputum cultures were positive; since completion of therapy, nineteen consecutive cultures have been negative over a period of almost a year.

Extensive examinations for tuberculosis have been performed on all of the mycologically proved cases of cavitary histoplasmosis. In all instances, more than thirty smears for acid-fast organisms were made and six or more cultures were performed. Concomitant tuberculous infection was found in approximately 20 per cent of this group.

Pathology: Anatomically, the changes found in cavitary histoplasmosis could not be distinguished either grossly or microscopically from tuberculosis. In other words, cavities and fibrosis dominated the gross picture; and epithelioid cell tubercles with giant cells, the microscopic picture. Yeast forms of *H. capsulatum* were occasionally demonstrable within macrophages on routine hematoxylin and eosin staining. More frequently special stains (periodic acid Schiff or Gridley) were required for the demonstration of the organism. Four of the cases in this series were diagnosed with the aid of pathologic examination. In all instances, the pathologic finding of typical intracellular yeast forms was confirmed by culturing *H. capsulatum* from the resected material. It is interesting to note that 4 additional cases not included in the mycologically proved group have been substantiated by the demonstration of the typical organism in tissues stained with periodic acid Schiff stain. These are 4 of 10 cases among the "serologically proved" cases who had pathologic material available for study when the serologic tests were found to be positive after surgical resection.

Serologically Proved Cases

Clinical, roentgenographic, and laboratory results: The designation "serologically proved" has been applied to those patients having serologic evidence of histoplasmosis but lacking cultural confirmation. It is believed that the majority of these patients would fall into the mycologically proved group if adequate studies were performed. The number of fungal cultures performed in the 97 "serologically proved" cases is presented in table 4. It is apparent that more than half of these cases had no cultures for fungi and only 20 per cent had more than two cultures. Several of those in which more than two sputum cultures were performed were in patients with nodular or miliary disease. Past experience with sputum cultures in these types of histoplasmosis has shown the yield of positive cultures to be small.

As a result of the screening techniques employed in the study, all forms of histoplasmosis were uncovered. In this group of 97 cases approximately 25 per cent had evidence of the more commonly recognized forms of histoplasmosis (14). The clinical manifestations presented by these patients were quite diversified, ranging from the asymptomatic pulmonary infiltration through the fatal acute disseminated form of the disease. Only one patient has died, and autopsy findings revealed disseminated histoplasmosis. Roentgenographic examination of this

[4] Eli Lilly and Co., Indianapolis, Indiana.

TABLE 4

NUMBER OF FUNGAL CULTURES DONE ON 97 SEROLOGICALLY PROVED CASES

Not done	54
1	16
2	9
3–5	10
6 or more	8

TABLE 5

COMPARISON OF SKIN TEST AND SEROLOGIC RESULTS AMONG 97 SEROLOGICALLY PROVED CASES OF HISTOPLASMOSIS

Serologic Test Positive	Skin Test Results					
	Histoplasmin			Tuberculin		
	Number Tested	Number Positive	Per Cent Positive	Number Tested	Number Positive	Per Cent Positive
By complement fixation.	61	43	70	74	53	71
By precipitin only.....	14	4	29	17	12	70

group of 24 cases revealed unresolved pneumonia in 40 per cent, nodular lesions in 35 per cent, and miliary infiltrations in 25 per cent of the cases. The remaining 73 "serologically proved" cases had apical or subapical disease, and approximately 60 per cent of them had either definite or suspected cavitary disease.

All of these patients had complement-fixing antibodies except for 17 instances in which only a positive precipitin test was demonstrated. The comparison of the results of serologic tests and skin tests on these 97 "serologically proved" cases is shown in table 5. Of the 80 patients with positive complement-fixation reactions, 74 were tested with tuberculin, 71 per cent of whom gave positive reactions. Sixty-one of the 80 were tested with histoplasmin, and 70 per cent gave a positive reaction. It is thus evident that, although the same percentage reacted to histoplasmin as in the mycologically proved cases (table 1), one-fifth more reacted to tuberculin. This suggests that the occurrence of both diseases in the same patient has probably been underestimated. Of the 17 patients who showed only a positive precipitin test, 70 per cent were tuberculin positive and only 29 per cent histoplasmin positive.

In table 6 are shown the results of the laboratory tests for tuberculosis among those "proved" by (*1*) positive precipitin test only, (*2*) complement-fixing antibodies only, and (*3*) isolation of *H. capsulatum*. Of the 17 patients in whom a positive precipitin test was the only finding suggestive of histoplasmosis, active tuberculous infection was demonstrated in 70 per cent. Among the 80 patients whose complement-fixation tests for histoplasmosis were positive, 52 per cent had laboratory evidence of tuberculosis. Among the 43 patients in whom infection was proved by isolation of *H. capsulatum*, 20 per cent had positive evidences of tuberculosis. The finding that half of the patients with positive complement-fixation tests had proved tuberculosis suggests that coexistent tuberculosis and histoplasmosis are probably much more frequent than was previously realized.

TABLE 6

RESULTS OF INVESTIGATIONS FOR TUBERCULOSIS IN SEROLOGICALLY AND CULTURALLY PROVED CASES

Proved	Positive Smear Only	Positive Culture Only	Both Smear and Culture Positive	Negative for Evidence of Tuberculosis	Per Cent with Positive Evidence of Tuberculosis
By precipitin only	6	2	4	5	70
By complement fixation only	14	4*	23	39	52
By isolation of *H. capsulatum*	0	2	7	34	20

* Two patients with positive urine culture, negative sputum.

DISCUSSION

Furcolow and Brasher (4) have estimated that probably some 1,200 patients with chronic cavitary histoplasmosis are occupying beds in tuberculosis hospitals located in central United States. The recognition and magnitude of this problem is of fundamental importance to all physicians concerned with the diagnosis and care of tuberculous patients. Since the clinical picture and roentgenographic findings are indistinguishable from those of tuberculosis, the clinician is forced to apply other tools to aid in distinguishing these entities.

The interpretation of the histoplasmin skin test is analogous to that of the tuberculin test, a positive reaction indicating either past or present infection. Present evidence indicates that once sensitivity has developed it is retained for many years and probably for life. Its usefulness as a clinical tool in the cavitary form of histoplasmosis, however, is shown to be somewhat limited. The finding that 26 per cent of the patients with proved histoplasmosis were nonreactors cannot be readily explained except that many are older persons and in a state of chronic debilitation. A few were acutely ill and might have been expected to be nonreactors. Of the seventeen cases of cavitary histoplasmosis reported in the literature, information on the histoplasmin skin test was available for thirteen. Three patients, or 23 per cent, were nonreactors. In spite of the high percentage of nonreactors, the demonstration of a positive reaction in a tuberculin-negative individual with cavitary disease offers strong evidence that the disease process is histoplasmosis. The real implication of the finding that one-quarter of the proved cases are skin-test negative is to alert physicians *not* to exclude the diagnosis on the basis of a negative skin test.

The complement-fixation test and the precipitin test have been used extensively as aids in diagnosis and in assessing the activity of the various forms of histoplasmosis. In the acute forms of the disease, precipitating antibodies are usually demonstrable prior to complement-fixing antibodies. It is believed that complement-fixing titers of 1:8 or greater indicate the presence of active disease. The potential value of the complement-fixation tests as a screen in assessing the activity of disease is indicated by the finding of serologic evidence for histoplasmosis in more than 85 per cent of the mycologically proved cases. Analysis of the serologic results in this study indicates that neither the complement-fixation test using histoplasmin nor the yeast phase antigen alone is sufficient. The number of

cases in which both complement-fixation tests were performed is too small to allow statistical evaluation of the relative value of the tests. Of the proved cases, approximately 60 per cent demonstrated a positive precipitin test in conjunction with positive complement-fixation titers. However, the finding of only 29 per cent skin test reactors to the histoplasmin skin test among the group having only a positive precipitin test leads to the belief that a positive precipitin alone offers insufficient evidence for presuming the diagnosis of chronic cavitary histoplasmosis

While the skin test and, in particular, the complement-fixation tests are useful screening tools, they are both secondary in importance to the demonstration of the etiologic agent. The present study indicates that in cases of cavitary histoplasmosis with productive cough, *H. capsulatum* may be readily and repeatedly demonstrated by sputum cultures. Although the finding of tubercle bacilli in the sputum may be sufficient to account for the pathologic process in an individual case, this finding should not exclude the possibility of concomitant histoplasmosis. The demonstration of serologic evidence of histoplasmosis in such a case should lead the clinician to a diligent search for this entity. Dual infections with the tubercle bacillus and *H. capsulatum* were found in 20 per cent of the cases studied. It is suggested that the true figure of dual infections is considerably higher. This concomitant infection with a fungus may serve to explain some of the so-called "treatment failures" in the chemotherapy of tuberculosis. Again emphasis should be laid on the fact that 15 to 20 per cent of the mycologically proved cases did not demonstrate a positive serologic test.

The necessity for pathologists to employ satisfactory special fungal stains in searching for Histoplasma cannot be overemphasized. The pathologic diagnosis of "chronic granuloma compatible with tuberculosis" in the absence of demonstrated acid-fast organisms appears no longer tenable. The demonstration of Histoplasma in 4 of 10 patients following surgery, about whom suspicion was aroused by the serologic results, is a case in point. Many of these patients had been discharged from the hospital months before a review of the slides was undertaken because of this study.

Summary

This paper attempts to summarize the usefulness and limitations of the laboratory tools available to establish the diagnosis of chronic progressive cavitary histoplasmosis. The data presented indicate that: The clinical and roentgenographic features of cavitary histoplasmosis are indistinguishable from those of cavitary tuberculosis. The skin test and the serologic tests for histoplasmosis are useful screening aids but each has its limitations. Approximately 25 per cent of known patients are nonreactors to the skin test. At least 10 per cent are negative by the serologic tests employed. In the light of these findings it is evident that a negative skin test or serologic test for histoplasmosis does not exclude the diagnosis of histoplasmosis.

Concomitant infections with *M. tuberculosis* and *H. capsulatum* are not uncommon and should be searched for. Such infections may account for some of

the treatment failures in the chemotherapy of tuberculosis. Mycologic studies of the sputum in proved cases reveal that *H. capsulatum* can be readily and repeatedly isolated in these cases. Adequate pathologic studies using special fungal stains are necessary to exclude a fungal etiology.

Sumario

Justipreciación de los Auxiliares Clínicos del Diagnóstico de la Histoplasmosis Cavitaria Evolutiva Crónica

Trátase aquí de sumarizar la utilidad y las limitaciones de los instrumentos con que cuenta el laboratorio para establecer el diagnóstico de la histoplasmosis cavitaria evolutiva crónica. Los datos presentados indican que las características clínicas y radiográficas de la histoplasmosis cavitaria son indistinguibles de las de la tuberculosis cavitaria. La cutirreacción y las serorreaciones para la histoplasmosis son auxiliares útiles para la tría, pero cada una de ellas adolece de sus limitaciones. Aproximadamente 25 por ciento de los enfermos conocidos no reaccionan a la prueba cutánea. A lo menos 10 por ciento resultan negativos con las serorreacciones empleadas. A la luz de estos hallazgos, es evidente que una cutireacción o serorreacción negativa para histoplasmosis no excluye el diagnóstico de esta dolencia.

No son raras las infecciones concomitantes por el bacilo tuberculoso y el *H. capsulatum* y hay que buscarlas. Esas infecciones pueden explicar algunos de los fracasos terapéuticos de la quimioterapia de la tuberculosis. Estudios micológicos del esputo en casos comprobados revelan que en ellos puede aislarse fácil y repetidamente el *H. capsulatum*. Para excluir una etiología micótica, se necesitan adecuados estudios histopatológicos con colorantes especiales para hongos.

Resume

Evaluation des moyens cliniques utilisables dans le diagnostic de l'Histoplasmose cavitaire chronique évolutive

L'auteur essaye de résumer l'utilité et les limites des moyens de laboratoire dont on dispose pour établir le diagnostic d'histoplasmose cavitaire chronique évolutive. Les données présentées indiquent que les aspects cliniques et radiologiques de l'histoplasmose cavitaire ne se distinguent pas de ceux de la tuberculose cavitaire. Les tests cutanés et les tests sérologiques pour la recherche de l'histoplasmose sont des moyens utiles, mais chacun d'eux a ses limites. Environ 25% des malades atteints d'histoplasmose ne réagissent pas au test cutané. Avec les tests sérologiques employés on a obtenu au moins 10% de résultats négatifs. A la lumière de ces résultats, il est évident que le test cutané ou sérologique négatif n'exclut pas le diagnostic d'histoplasmose.

Les infections concomitantes par le bacille tuberculeux et par *H. capsulatum* ne sont pas rares et doivent être recherchées. Ces infections permettent d'expliquer certains échecs de la chimiothérapie de la tuberculose. Dans ces cas, l'étude mycologique de l'expectoration dans les cas d'histoplasmose démontrée révèle que l'isolement facile et répété de *H. capsulatum* est de règle. Des études histologiques adéquates avec colorants spéciaux pour le fongus sont nécessaires pour pouvoir exclure l'étiologie fungique.

Acknowledgment

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REFERENCES

(1) Johnson, H. E., and Batson, R.: Benign pulmonary histoplasmosis: A case report with a brief review of the literature, Dis. of Chest, 1948, *14*, 517.

(2) Bunnell, I. L., and Furcolow, M. L.: A report on ten proved cases of histoplasmosis, Pub. Health Rep., 1948, *63*, 299.

(3) Sutliff, W. D., Hughes, F., Ulrich, E., and Burkett, L. L.: Active chronic pulmonary histoplasmosis, A.M.A. Arch. Int. Med., 1953, *92*, 571.

(4) Furcolow, M. L., and Brasher, C. A.: Chronic progressive (cavitary) histoplasmosis as a problem in tuberculosis sanatoriums, Am. Rev. Tubere., 1956, *73*, 609.

(5) Furcolow, M. L.: Further observations on histoplasmosis mycology and bacteriology, Pub. Health Rep., 1950, *65*, 965.

(6) Hodgson, C. H., Weed, L. A., and Clagett, O. T.: Pulmonary histoplasmosis: Review of published cases and report of an unusual case, J. Thoracic Surg., 1950, *20*, 97.

(7) Hodgson, C. H., Weed, L. A., and Clagett, O. T.: Pulmonary histoplasmosis: Summary of data on reported cases and a report on two patients treated by lobectomy, J.A.M.A., 1951, *145*, 807.

(8) Forsee, J. H., Puckett, T. B., and Hagman, F. E.: Surgical considerations in focalized pulmonary histoplasmosis, J. Thoracic Surg., 1953, *26*, 131.

(9) Peeples, W. J., and Spence, M. J.: Pulmonary cavitation due to *Histoplasma capsulatum*, Am. Rev. Tubere., 1954, *69*, 111.

(10) Monroe, J., and Kurung, J. M.: Histoplasmosis, with review of the literature and report of a case, proved by culture, with involvement of the upper lobe of each lung simulating active bilateral apical pulmonary tuberculosis, Ann. Int. Med., 1953, *38*, 206.

(11) Salvin, S. B., and Furcolow, M. L.: Precipitins in human histoplasmosis, J. Lab. & Clin. Med., 1954, *43*, 259.

(12) Charr, R.: Histoplasmosis: Report of two cases, Am. Rev. Tubere., 1953, *67*, 376.

(13) Polk, J. W., Brasher, C. A., de Castro, J., and Buckingham, W. W.: The surgical treatment of pulmonary histoplasmosis with an evaluation of MRD-112 as a possible adjunct, J. Thoracic Surg., 1956, *31*, 148.

(14) Furcolow, M. L.: The clinical diagnosis of histoplasmosis, Postgrad. Med., 1956, *20*, 349.

DEMONSTRATION OF CIRCULATING ANTIBODIES TO M. TUBERCULOSIS IN HUMANS[1]

I. Technique. Theoretical Considerations and Development of a New Concept of Antibody Assay as Applied to Tuberculosis

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INTRODUCTION

The demonstration of the existence and significance of circulating antibodies to tuberculosis would further the understanding of the disease process. Through the years, the problem has been complicated by the diversity of antigenic components that is possible with the mycobacteria.

Serologic procedures have been developed which were correlated with the disease state. Such methods as the complement-fixation test (1) and the hemagglutination test (2) have been used, but have not been found to be practicable in the clinical laboratory. A flocculation test (3) has also been reported, but still requires study. The various skin-testing procedures appear to measure tissue hypersensitivity to tuberculoprotein and, as such, may be of only limited value in given cases. The skin hypersensitivity phenomenon theoretically demonstrates a tissue-bound antibody reaction rather than a reaction with circulating antibody.

Several years ago, two of the present writers (S. M. D. and M. H. F.) worked with Coombs serum in an attempt to demonstrate agglutination of attenuated *Mycobacterium tuberculosis* in the presence of serum from patients with active tuberculosis. Under the best experimental circumstances, the normal clumping of the mycobacteria interfered with the interpretation of specific agglutination. It was then decided that an indirect approach would be more feasible.

Recently, Weiner (4) reported a serologic method for gamma globulin determination using Coombs serum. Since the globulin fraction of serum contains antibody activity and since Coombs serum contains potent anti-human globulin, a method for the indirect determination of antibody globulin adsorbed to a particulate antigen appeared feasible (figure 1).

This report deals with experimental trials when serum from normal subjects and from patients with active tuberculosis was adsorbed by a polyvalent antigen, the antigen-antibody complex was treated with Coombs serum, and the residual Coombs serum was titrated with maximally coated erythrocytes. Experimental application of the technique to a large series of patients is the subject of the subsequent report (5).

[1] From the Department of Pathology, U. S. Army Hospital, Fort Benning, Georgia; and the Section for Infectious Diseases, Department of Laboratories, D. C. General Hospital, Washington, D. C.

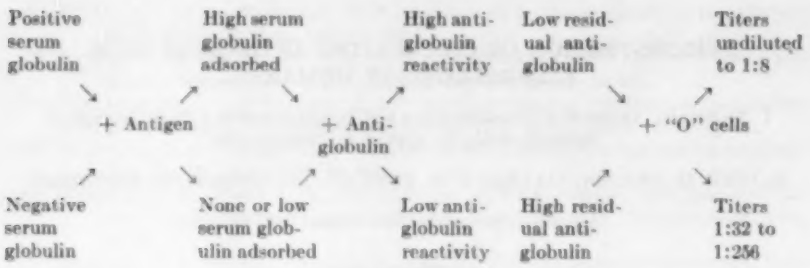

FIG. 1. Flow sheet of test procedure.

METHODS AND MATERIALS

M. tuberculosis antigen was prepared by pooling organisms isolated from active cases of pulmonary tuberculosis. These organisms were grown on Petragnani medium in 120 ml. screw-cap prescription bottles for a period of four weeks. The organisms were killed by placing enough 10 per cent buffered formalin in the culture bottle to cover the slant. After twelve hours, the organisms were loosened from the medium by a wire loop. The formalin suspension containing the *M. tuberculosis* was then poured into bottles containing glass beads and was forcefully shaken to produce a fairly uniform suspension. The organisms were then washed four times in physiologic saline and finally were resuspended in physiologic saline in a concentration matching the No. 4 McFarland turbidity standard.

Maximally coated O-Rh-positive human erythrocytes were prepared by washing the cells three times in physiologic saline. Then the packed washed erythrocytes were resuspended in an equal volume of Anti-D slide-test serum and were incubated for one hour at 37°C. These cells were again washed three times in physiologic saline and were resuspended in physiologic saline to a 2 per cent concentration.

Five milliliters of the serum to be tested were employed. (The serum must be recently drawn because the antibody reaction fails to occur in this system when sterile serum is stored at 4°C. for more than forty-five days.) Antigen excess was determined by testing multiple concentrations of antigen against fixed volumes of serum. When the excess concentration of antigen was determined, this concentration was represented by the organisms contained within 15 ml. of antigen suspension. This amount of antigen was centrifuged for twenty minutes at 3,000 r.p.m. The bacterial cells were washed three times and the supernatant was discarded. The serum to be tested was then used as a suspending medium for the bacteria. It was shaken and incubated overnight at room temperature. After incubation, the incubation mixture was centrifuged for twenty minutes at 3,000 r.p.m. and the super-

TABLE 1

TECHNIQUE FOR "BACK-TITRATING" COOMBS SERUM

Tubes	1	2	3	4	5	6	7	8	9	10
Saline		0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Supernatant from Coombs *Mycobacterium tuberculosis* mixture	0.2	0.2	Mix and transfer serially from 2 through 10							
Maximally coated "O" Rh-positive cells	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Final titer of serum	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512

natant serum was discarded. The antigen-antibody complex was washed three times with saline and the supernatant was discarded each time. The complex was then suspended in 1 ml. of Coombs serum and incubated for two hours at room temperature. After incubation and centrifugation, 0.4 ml. of the Coombs serum was withdrawn and titrated (table 1). Originally, the test was performed by centrifuging for one minute at 500 r.p.m., and the agglutination inhibition was read.

Reading of the test was simplified when it was found that, if the tests were allowed to settle overnight instead of being spun for one minute at 500 r.p.m., the cells which were agglutinated would form a uniform button of erythrocytes. When agglutination inhibition was apparent, the settled cells were in a scatter pattern.

A control for the Coombs serum is set up identically as in table 1. The usual titer was 1:256 to 1:512, depending on the lot of the Coombs serum used. All test results were interpreted to a standard reference titer of 1:256.

TABLE 2

RESULTS OF TESTS WITH NORMAL SERUM USING VARYING AMOUNTS OF ANTIGEN

Test	Serum Titer	Antigen Employed
		ml.
1	1:16	5
2	1:32	5
3	1:32	5
4	1:32	5
5	1:64	15
6	1:32	15
7	1:32	15
8	1:32	15
9	1:32	15
10	1:32	15

TABLE 3

RESULTS OF TESTS OF SERUM FROM PATIENTS WITH ACTIVE TUBERCULOSIS USING VARYING AMOUNTS OF ANTIGEN AND VARYING INCUBATION TIME OF COOMBS SERUM

Test	Serum Titer	Antigen Employed	Remarks
		ml.	
1	1:2	5	
2*A*	1:64	2.5	
B	1:8	5	Same serum as 2*A* 5 ml. antigen
C	Undiluted	10	Same serum as 2*A* 10 ml. antigen
3	1:2	15	
4	Undiluted	15	
5	1:2	15	
6*A*	1:8	15	Coombs titration incubated at room temperature two hours
B	1:8	15	Same as 6*A* Coombs titration incubated one hour at room temperature
C	1:8	15	Same as 6*A* Coombs titration incubated thirty minutes at room temperature

Results

Typical results, obtained when the antigen concentration was varied and normal serum was tested, are demonstrated in table 2. It will be noted that significant differences were not apparent with normal serum when the antigen was varied. However, this factor became pronounced when serum from patients with active tuberculosis was tested (table 3). Experimental results indicated that 10 ml. of antigen suspension appeared to be the minimal required amount. It was then decided that 15 ml. of suspension would provide a uniform excess, as illustrated by other serum.

Specimens *A*, *B*, and *C* (table 3) are specimens of the same serum in which the incubation time was varied with respect to the Coombs serum. It may be seen that the thirty-minute incubation was as effective as the two-hour incubation.

Discussion

The results of the test indicate that more antibody globulin is adsorbed on tubercle bacilli from serum from subjects with active tuberculosis than from serum from subjects without active tuberculosis.

It should be emphasized that this test measures the adsorbed antibody globulin by assaying the globulin reactivity of the adsorbed molecule. Thus, the test is a measure of the amount of globulin adsorbed to the particulate antigen and is not necessarily a measure of incomplete or blocking antibodies only.

In a series of 20 tests of normal serum regardless of the skin sensitivity of the individual, serologic findings were negative. The test, therefore, does not appear to be correlated with the skin hypersensitivity reaction. In fact, the test appears to correlate only with active tuberculosis when *M. tuberculosis* is used as the adsorbing antigen.

Further experimentation will be required to determine the limits of sensitivity. It seems likely that this method could be applied as a means of demonstrating antibody globulin in any disease in which a particulate antigen capable of adsorption of antibody could be prepared. Cohn (6) has been studying adaptation of the technique to the study of amebiasis. Further studies will be presented on a large series of cases to elucidate the clinical potentialities of the test.

Summary

A method for the demonstration of circulating antibodies to *M. tuberculosis* is presented. The data indicate that the antibody globulin measured was related to the active disease state but was unrelated to the skin test. This method appears applicable to bacterial or other diseases in which a particulate antigen can be prepared.

Sumario

Descubrimiento de Anticuerpos del M. Tuberculosis en la Circulación de Seres Humanos: I. Técnica. Consideraciones Teóricas y Elaboración de un Nuevo Concepto de la Valoración de Anticuerpos Aplicado a la Tuberculosis

Se presenta un método para el descubrimiento de anticuerpos circulantes del *M. tuberculosis*. Los datos indican que la globulina de los anticuerpos medida se relacionaba con el estado de enfermedad activa, pero no con la cutirreacción. Este método parece aplicable a las afecciones bacterianas o de otro género en que puede prepararse un antígeno en partículas.

Resume

Démonstration d'anticorps de M. tuberculosis dans la circulation chez l'homme: I. Technique. Considérations théoriques et élaboration d'une nouvelle méthode pour l'évaluation des anticorps dans la tuberculose

Présentation d'une méthode pour la démonstration d'anticorps de *M. tuberculosis* dans la circulation. Les données obtenues dans la détermination de la globuline des anticorps indiquent qu'elle est en rapport avec la maladie en activité, mais sans corrélation avec le test cutané. Cette méthode paraît applicable à d'autres affections bactériennes ou d'étiologie différente dans lesquelles un antigène spécifique peut être obtenu.

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REFERENCES

(1) Pinner, M.: Complement fixation in tuberculosis: III. Studies on the nature of the antigen, Am. Rev. Tuberc., 1925, *12*, 142.

(2) Middlebrook, G., and Dubos, R.: Specific serum agglutination of erythrocytes sensitized with extracts of tubercle bacilli, J. Exper. Med., 1948, *88*, 521.

(3) Raab, W.: Microflocculation test for tuberculosis, Am. J. Clin. Path., 1956, *26*, 327.

(4) Weiner, A. S.: Serologic test for gamma globulin, Am. J. Clin. Path., 1955, *25*, 595.

(5) Fusillo, M. H., Weiss, D. L., and Dozier, S. M.: Demonstration of circulating antibodies to *M. tuberculosis* in humans: II. Clinical laboratory studies, Am. Rev. Tuberc., 1957, *75*, 954.

(6) Cohn, R.: Personal communication.

DEMONSTRATION OF CIRCULATING ANTIBODIES TO M. TUBERCULOSIS IN HUMANS[1]

II. Clinical Laboratory Studies. The Existence of Specific Circulating Antibody in Patients with Active Tuberculosis

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Introduction

Dozier, Fusillo, and Woodham (1) have recently introduced a technique for the demonstration of circulating antibodies to *M. tuberculosis* in patients with active tuberculosis. The technique was based on the theory that specific antibody globulin is extracted from serum by an antigen-antibody reaction with a polyvalent *M. tuberculosis* antigen. The adsorbed antibody was then assayed by a fall in titer of Coombs serum when human "O" Rh-positive cells sensitized with Anti-D serum were used as the indicator system. The present report will elaborate on experiences with the latter method, as applied to the study of tuberculosis antibody, in the sera of 178 normal subjects, patients with active tuberculosis, personnel continually exposed to tuberculosis, and persons with other chronic upper respiratory infections.

Methods and Materials

The formalin-killed antigen was prepared by the methods previously described (1). A thick suspension of bacteria was produced by shaking with glass beads in a mechanical shaker. The suspension was then made up to give 20 per cent transmission on a photoelectric colorimeter at 540 A. The amount of bacteria contained in 15 ml. of this suspension was then used as the antigen for the adsorption of antibody. Readings were made by the modification previously noted (1).

Serum was collected from patients with active tuberculosis who were selected on the basis of clinical, roentgenographic, and bacteriologic evidence. The sera from these patients, as well as from the exposed hospital personnel without tuberculosis, were obtained from the Pulmonary Disease Division of the D. C. General Hospital. Other sera were obtained from laboratory workers, other healthy normal subjects, and from patients suffering from various other diseases, as noted in the tables.

Results

The following data demonstrate the presence or absence of circulating antibodies to *M. tuberculosis* in the sera of 178 persons among the various groups tested. It may be seen in table 1 that, among 41 normal subjects tested, the serum titers ranged from 1:32 to 1:256. This would indicate that a limited surface activity of adsorbed globulin may be demonstrated in some normal persons. In contrast, the 76 patients with active pulmonary tuberculosis showed a marked fall in titer (undiluted to 1:8). One patient with tuberculosis of the

[1] From the Section for Infectious Diseases, Department of Laboratories, D. C. General Hospital, Washington, D. C.; and U. S. Army Hospital, Fort Benning, Georgia.

hip gave a titer of 1:8. Of patients with tuberculous meningitis whose sera were tested, 2 gave false negative results of 1:128 and a third, a titer of 1:8.

The results of serum titers of a total of 33 hospital personnel who are constantly exposed to active tuberculosis are given in table 2. As was shown among the hospital employees group, all workers with the exception of one resident showed titers in the normal range. Moreover, among the pathologists, except for 2 "false positive" reactions which were negative on re-testing, the titers were all in the normal range.

TABLE 1

SERUM TITERS FOR CIRCULATING ANTIBODY TO M. TUBERCULOSIS IN NORMAL SUBJECTS AND PATIENTS WITH ACTIVE TUBERCULOSIS

Group	Number Tested	Serum Titers
Normal	41	1:32 to 1:256
Active tuberculosis	80	
Pulmonary	76	Undiluted to 1:8
Hip bone	1	1:8
Meningitis	1	1:8
	2*	1:128

* Apparently false negatives (*see* text).

TABLE 2

SERUM TITERS FOR CIRCULATING ANTIBODY TO M. TUBERCULOSIS IN NORMAL SUBJECTS CONSTANTLY EXPOSED TO ACTIVE DISEASE

Group	Number Tested	Serum Titers
Normal exposed	33	
Tuberculosis hospital employees	26	1:64 to 1:256
	1*	1:2
Pathologists	6	
	2*	*1:2, 1:8; †1:64
	4	1:64 to 1:128

* Subjects had upper respiratory infections two weeks prior to testing.

† Repeat of test six to eight weeks after first test.

TABLE 3

SERUM TITERS FOR CIRCULATING ANTIBODY TO M. TUBERCULOSIS IN 7 CASES OF ARRESTED TUBERCULOSIS

Time Since Last Positive Findings	Number of Cases	Serum Titers
7 months	1	1:64
8 months	1	1:32
9 months	2	1:64; 1:128
12 months	1	1:64
2 years	1	1:128
5 years	1	1:256

TABLE 4

SERUM TITERS FOR CIRCULATING ANTIBODIES TO M. TUBERCULOSIS OF INFECTIOUS DISEASES OTHER THAN TUBERCULOSIS

Group	Number Tested	Serum Titers
Respiratory diseases	13	
Chronic	6	1:64 to 1:128
Sarcoidosis	2	1:64
Adenoviruses	5*	1:256
Immune globulin	1	1:256
Typhoid	1	1:64

* Includes one pooled high titer control adenoviral serum.

The antibody titers in 7 cases of arrested tuberculosis are listed in table 3. Titers in the normal range were obtained in 4 cases; and a "borderline" titer, in one case. These examinations covered a period from seven to twelve months after the last sputum or gastric culture for *M. tuberculosis* had been obtained.

In table 4 the serum specimens obtained from other diseases are classified. It was demonstrated that serum titers within normal limits were obtained from patients with chronic upper respiratory disease (including asthma), sarcoidosis (2 cases), and viral respiratory infections (5 cases including one serum positive for adenovirus from a control subject). Immune globulin, as well as the serum from one case of typhoid, showed no antibodies for *M. tuberculosis*.

DISCUSSION

It may be seen from the foregoing tables that, when normal serum and serum from patients with active tuberculosis are compared, a distinct difference in the titers of adsorbed antibodies is observed. The results are very clear cut. There is at least a three-tube difference in the titration results between serum from cases of active tuberculosis and from persons without active disease.

There is a suggestion that circulating antibody levels are reduced to normal levels in clinically arrested tuberculosis (table 3). In the one borderline "normal titer" serum, the patient was scheduled to be discharged within sixty days after the test.

Among the 13 cases of nontuberculous upper respiratory infections, including one pooled serum, all gave titers in the normal range.

Thus, in the total of 178 cases presented, there were 3 "false positives" and 2 apparently "false negative" tests. In the 2 pathologists who showed "false positive" titers, serum was retested eight weeks after the first test and showed normal titers. In the group of 27 tuberculosis hospital employees tested, the serum from one resident physician also demonstrated a "false positive" reaction. It was established that all 3 of these subjects had suffered mild upper respiratory infections within a couple of weeks prior to the testing of the serum. It was then considered that either a cross-reacting entity was involved or that perhaps constant exposure to tuberculosis might produce a subclinical infection or transient antibody levels. It is also possible that the appearance of false

positive results may not be based on specific antibody globulin at all, but rather upon a nonspecific surface Coombs activity. It is recognized that care must be taken in the evaluation of any Coombs serum reactions.

A few scattered inconclusive results of testing BCG-immunized persons have led the present writers to make plans for a comprehensive study, using the next group of medical students who will be immunized at local universities.

The recognition of preceding upper respiratory infections in the 3 patients with false positive reactions prompted the study of serum with known high titers of adenovirus antibodies which, as listed in table 4, were shown to be in the normal range.

Studies with concentrated immune globulin could help exclude cross reactivity with antibodies to influenza *B*, mumps, typhoid, paratyphoid *A* and *B*, Coxsackie *A*, and adenovirus. The latter is based on the presence of these specific antibodies in the typical immune serum tested.

With respect to the meningitis serum tested, only one of 3 specimens showed circulating antibody. The other 2 at the present time must be considered false negatives. One source of possible error may be that the sera were lysed when they reached the laboratory and that antigens contained therein may have given nonspecific agglutination. All 3 children, however, had positive cerebrospinal fluid findings and positive skin tests. The case of tuberculosis of the hip was diagnosed by clinical findings.

These studies further confirm the original observations that the technique reported measures a circulating antibody irrespective of the tissue hypersensitivity state of the subject tested.

While the antigen may be considered crude, modification and extraction may be shown to be feasible with further study. An outstanding feature of the method is that it is readily adaptable to the laboratory production of antigens from other bacteria or fungi from which it has formerly been difficult to produce antigens. Recent information indicates the adaptability of the method to the study of amebiasis (2). Deterioration of the specific antibody globulin in serum forty-five days after refrigeration might be a clue to the type of antibody being measured. The latter may also account for the complete lack of specific antibody in immune globulin by this method of testing. The relationship of the antibody to fractions of antigens of *M. tuberculosis* var. *hominis* as well as to the avirulent mycobacteria must be further explored. Experimental studies to describe the significance of a rise and fall in antibody titer are also warranted.

Acknowledgments

The writers wish to express their appreciation to Dr. Sol Katz, Director, Pulmonary Disease Division; Dr. Marc West, Pediatrics Division; and the staff of the Medical Services who kindly made material available for the current study. Dr. Edward Marshall and Mrs. Patricia Behrens rendered valuable assistance in phases of the project.

REFERENCES

(1) Dozier, S. M., Fusillo, M. H., and Woodham, G.: Demonstration of circulating antibodies in humans: I. Technique, Am. Rev. Tuberc., 1957, *75*, 949.

(2) Cohn, R.: Personal communication.

SEROLOGIC INVESTIGATIONS OF MYCOBACTERIAL ANTIGENS[1, 2]

V. The Influence of Normal Serum Components on the Sensitization and Reactivity of Antigen-Treated Sheep Erythrocytes

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(Received for publication December 18, 1956)

INTRODUCTION

Previous reports from the laboratories in which the present study was made have been concerned with the serologic characterization of erythrocyte-adsorbable antigens obtained from a single bacillary mass of BCG by successive aqueous extractions (1–4). Antigenic specificity is gained with succeeding extractions as shown by the failure of antimycobacterial serums, other than anti-BCG, to react with the extract-treated erythrocyte suspensions in hemolytic tests.

During the course of the present studies, the question was raised as to the influence of normal serum or normal serum components on the relationship between BCG extract or Old Tuberculin (OT) and sheep erythrocytes. This seemed especially pertinent since it already has been established that differences, other than those of specificities, exist between BCG extract-treated and OT-sensitized erythrocytes. For example, antigens present in OT and in BCG extract are adsorbed to different sites on the sheep erythrocyte (4). In addition, while OT-sensitized erythrocytes demonstrate agglutination or hemolysis under appropriate conditions, BCG extract-treated erythrocytes rarely exhibit the former reaction.

The data to be described are the results of experiments designed to determine whether or not normal serum (or serum components) would interfere with the reactivity between test serums and sheep erythrocytes previously treated with BCG extract or with OT. Also examined was the effect of serum on the mechanism of erythrocyte sensitization by these two antigen preparations.

MATERIALS AND METHODS

Sensitization of sheep erythrocytes with BCG extracts and with OT[3]: The method employed for obtaining BCG extracts for the specific sensitization of sheep erythrocytes has been described previously (1), as has the procedure utilized for treating erythrocytes with OT (5).

Hemagglutination and hemolytic tests: The details for performing the hemagglutination and hemolytic tests have been reported from these laboratories as "drop" modifications of more conventional procedures (1, 5). All serums are heat-inactivated at 56° C. for thirty minutes, followed by adsorption with washed, normal sheep erythrocytes to remove naturally occurring antibodies. Two drops of each progressive doubling serum dilution

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[1] From the Department of Bacteriology, The Ohio State University; and from the Ohio Tuberculosis Hospital, Columbus, Ohio.

[2] This study was supported in part by a grant from the Ohio Tuberculosis and Health Association.

[3] Old Tuberculin (4X International Std.) was generously provided by Dr. W. S. Hammond, Lederle Laboratories Division, American Cyanamid Corporation, Pearl River, New York.

(prepared in saline buffered with phosphates to pH 7.4) are mixed with 2 drops of a 0.5 per cent suspension of previously sensitized sheep erythrocytes. To each tube in the hemolytic series is added 1 drop of guinea pig complement from which antibody to sheep erythrocytes has been removed by adsorption in the cold. The complement is introduced as a 1:9 saline dilution. Both the hemolytic and the hemagglutination tests are incubated at 37°C. (water bath) for thirty minutes. After light centrifugation the hemagglutination titer is determined as the last serum dilution exhibiting definite aggregation. The hemolytic titer is determined by the last serum dilution demonstrating complete hemolysis.

Serums: Anti-BCG and anti-*Mycobacterium bovis* serums were recovered from blood samples obtained by cardiac puncture of adult rabbits which had received 2.0 mg. of inoculum of either organism intravenously.

Normal human serums were obtained from blood specimens made available through the clinical laboratories of the Ohio Tuberculosis Hospital.

Serum fractions and fractionation procedure: The following commercially available serum fractions were employed in this study:

Bovine albumin (30 per cent solution)[4]
Normal human albumin (salt poor)[4]
Poliomyelitis immune globulin (human)[4]

As indicated in the text (below), these products were diluted to concentrations corresponding to those present in normal serum. In some instances it was found necessary to add dry NaCl to achieve isotonicity.

Rabbit and some human serum fractions were prepared by Na_2SO_4 precipitation (6) and adjusted to concentrations comparable with those reported for normal serum.

Experimental Results

Influence of normal serums on reactivity of BCG extract-treated and OT-sensitized sheep erythrocytes: Each of the sensitized erythrocyte suspensions (0.5 per cent) was treated with normal rabbit serum and was subsequently tested for reactivity with anti-BCG serum in the following manner:

Two drops of either BCG extract-treated sheep erythrocytes or OT-sensitized erythrocytes were mixed with 2 drops of normal rabbit serum and were incubated at 37°C. (water bath) for thirty minutes. The erythrocytes were washed free of the normal rabbit serum by repeated centrifugations in the presence of excess saline (pH 7.4) and were finally resuspended to the original 2 drops. Two drops of a known anti-BCG serum (four hemolytic units) were mixed with the erythrocyte preparations in the presence of 1:9 guinea pig complement (1 drop) and the mixtures were reincubated for thirty minutes at 37°C. Saline, substituted for serum, served to control the influence of the additional incubation period on the antigenicity of the sensitized erythrocyte suspensions.

The results indicated that prior treatment of the BCG extract-treated erythrocytes with the normal rabbit serum subsequently interfered with the lysis of these erythrocytes by the anti-BCG serum. The reactivity of the OT-sensitized erythrocytes was unaffected by the treatment with the normal serum since hemolysis was observed with the test anti-BCG serum.

The experiment was repeated with normal human serum with identical results.

By substituting saline dilutions of the normal rabbit and human serums (1:2 to 1:256) for the undiluted serums in the above test, the titer of the serum fac-

[4] From the Armour Laboratories, Chicago, Illinois.
[5] From the Cutter Laboratories, Berkeley, California.

TABLE 1

TITRATION OF SERUM FACTOR RESPONSIBLE FOR MODIFYING ANTIGENICITY OF BCG EXTRACT-SENSITIZED ERYTHROCYTES

Sheep Erythrocytes Sensitized with:	Control: 4 Hemolytic Units of Anti-BCG Serum		Titer of "Serum Factor"							
			1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
OT, 1:15	+*	Normal human serum	+	+	+	+	+	+	+	+
		Normal rabbit serum	+	+	+	+	+	+	+	+
BCG, extract (H_2O)	+	Normal human serum	−	−	−	−	−	−	−	+
		Normal rabbit serum	−	−	−	−	−	−	−	+

* + indicates hemolytic demonstration

tor responsible for interfering with the reactivity of the BCG extract-treated erythrocytes and homologous serum was determined. As shown in table 1, 1:128 dilutions of either normal serum completely prohibited the lytic demonstrations.

However, if the BCG extract-treated erythrocytes were mixed first with a known positive serum, incubated and washed, the re-suspended cells were subsequently lysed upon the addition of 1:9 complement, despite the introduction of normal rabbit serum into the systems.

Elutability of BCG extract antigens from sheep erythrocytes in the presence of normal serum: The above observations, particularly the latter, suggested that a factor or factors naturally present in serum might remove the BCG antigens from erythrocytes rather than function as inhibitors of antigen-antibody reaction. This elution does not occur, however, if the extract-treated erythrocytes are first allowed to react with a serum containing an adequate level of antibody, probably due to a "binding" or "fixing" action of the antibody. In other words, a competition apparently exists between the antibody content of a given serum (reactivity) and its antigen-eluting property. A similar phenomenon was not found to be associated with OT-sensitized erythrocytes.

To test this hypothesis, BCG extract-treated sheep erythrocytes were mixed with normal rabbit serum, washed, tested with a positive serum, re-washed, tested again with the positive serum, re-washed, sensitized with fresh BCG extract, and re-tested with the anti-BCG serum.

This experiment and the observed results are diagramed in figure 1. The eluting property of rabbit serum is quite marked, since extract-treated cells subsequent to treatment with this serum behaved as did normal sheep cells and could be re-sensitized with fresh BCG extract. Other experiments revealed that normal human serum also possessed the same property. By way of comparison, phosphate-buffered saline (pH 7.4) failed to remove the extract antigen(s) from the sheep erythrocytes.

BCG Extract-Sensitized Sheep Erythrocytes

↓

Centrifuged and washed 3 times with buffered saline. Supernatant fluids discarded

1.0 ml. of normal rabbit serum (previously adsorbed with normal sheep erythrocytes) added to 0.1 ml. of extract-treated erythrocytes. Incubation 30 minutes at 37°C.	1.0 ml. of saline (Same)
Erythrocytes recovered, washed, and re-suspended to 0.5%	(Same)
Erythrocyte suspension tested with anti-BCG serum. Hemolytic titer—*negative*	(Same—Hemolytic titer—*1:64*)
Erythrocytes recovered as before and re-sensitized with fresh BCG extract	(Same)
Tested with anti-BCG serum. Hemolytic titer—*1:32*	(Same—Hemolytic titer—*1:32*)

FIG. 1. Diagram of experiment demonstrating the elutability of BCG extract antigens from sheep erythrocytes by treatment with normal rabbit serum.

TABLE 2

INHIBITION OF SHEEP ERYTHROCYTE SENSITIZATION WITH OT AND BCG EXTRACT BY SERUM ALBUMIN

Erythrocyte-Sensitizing Antigen	Diluent* for Erythrocyte-Sensitizing Antigen		Titers†			
	Human Albumin	Human Globulin	Anti-BCG		Anti-*M. Bovis*	
			H. L.	H. A.	H. L.	H. A.
OT, 1:15 (saline)			1,024	128	32	8
	1:16		64	8	—	—
	1:32		128	16	—	—
	1:64		256	32	4	—
	1:128		256	64	16	—
	1:256		1,024	128	32	4
		1:8	1,024	128	32	8
		↓	↓	↓	↓	↓
		1:256	1,024	128	32	8
BCG extract (saline)			64	—	—	—
	1:16		—	—		
	1:32		—	—		
	1:64		—	—		
	1:128		16	—		
	1:256		32	—		
	1:512		64	—	—	—
		1:8	64	—	—	—
		↓	↓	↓	↓	↓
		1:256	64	—	—	—

* Albumin and globulin fractions initially adjusted to concentrations found in normal serum

† All titers expressed as reciprocals of serum dilutions: H.L. refers to hemolysis; H.A. to hemagglutination

Serum fraction responsible for removal of extract antigen(s) from sheep erythrocytes: Both albumin and globulin fractions from several species (human, rabbit, and bovine) were examined to determine in which serum fraction or fractions the property of "antigen removal" resided. After restoring isotonicity to several of the protein preparations by the addition of dry NaCl and adsorbing the globulins with normal sheep erythrocytes, experiments similar to the one just described were undertaken. In all instances the albumins proved the offending fractions. As before, OT-sensitized erythrocytes were unaffected.

Serum fraction interfering with sensitization of sheep erythrocytes by OT and BCG extract: Several investigators (7, 8) have either stated directly or alluded to the fact that sensitization of erythrocytes with OT is inhibited by serum. The findings just reported suggested that albumin might be the component of serum responsible for inhibition. The results (table 2) of an experiment designed to test the capacity of OT and BCG extract to sensitize sheep erythrocytes in the presence of human albumin and globulin confirmed this supposition. Each of the serum fractions was initially adjusted by dilution in saline to correspond to its normal serum concentration. The sensitization of erythrocytes by either OT or BCG extract is inhibited either entirely or in part by the presence of albumin in dilutions as great as 1:128.

Globulin is without influence on the sensitizing reaction.

Discussion

The elutability of the BCG extract antigens from the sheep erythrocyte surface by normal serum or albumin, in the absence of sufficient antibody, reflects the character of the antigen-erythrocyte union. With reference to the current studies, this observation serves further to distinguish these antigens from the erythrocyte-adsorbable antigens contained in OT. Whether or not one can project this property of elutability to explain the singular specificity of the BCG antigens is being considered. Perhaps the failure of antimycobacterial serums, other than anti-BCG, to react with extract-treated erythrocytes may be attributable not to the complete absence of potentially reactive antibody in these serums, but to the eluting property of the serum albumin in the presence of inadequate antibody levels. In any event, the union of antibody with BCG extract-treated erythrocytes sufficiently alters the antigen-erythrocyte surface relationship so that exposure to albumin does not result in antigen elution.

A number of investigators have described the inhibiting role of lipids on adsorption of bacterial antigens to erythrocytes. Although the majority of these studies has been concerned with antigens other than those obtained from mycobacteria (9–11), Boyden and Grabar (12) found lipids to interfere with the sensitization of erythrocytes by constituents of tuberculin. Fine and associates (7) reported that certain serums from ill persons, including those hospitalized with tuberculosis, and some serums from normal persons prevented the sensitization of sheep erythrocytes by OT. Comparative electrophoretic patterns, total serum protein determinations, and albumin-globulin ratios of the inhibiting and noninhibiting serums did not reveal the identity of the interfer-

ing factor. These workers, however, made no attempt to determine in which serum fraction or fractions the inhibitor resided. Boyden and Anderson (8) have shown that serum albumin inhibits, although not completely, the agglutination of normal sheep cells by a mixture of selected antimycobacterial rabbit serum and tuberculoprotein.

From the data reported above, the role of albumin in preventing erythrocyte sensitization by OT is quite evident and to some extent confirms the conclusions of Boyden and Anderson although the experiments were designed differently.

Whether or not this property of albumin can be translated to explain the inability to sensitize rabbit erythrocytes by intravenous injection of moderate quantities of OT is problematic. These and related experiments will be reported later. Boyden (13) was able to demonstrate tuberculopolysaccharide on the erythrocytes from guinea pigs after three hours and up to six days following injection. However, it was necessary to employ large quantities of the tuberculopolysaccharide (10–20 mg.).

Summary

BCG extract antigens are eluted from the sheep erythrocyte by normal serum (rabbit or human). The eluting factor was shown to be associated with the albumin fractions. Bovine albumin possesses a similar property. Old Tuberculin (OT)-sensitized sheep cells are unaffected.

The interaction of BCG extract-treated erythrocytes and homologous antibody prevents elution of the complex from the erythrocyte upon subsequent exposure to normal serum.

Sensitization of sheep erythrocytes by OT and BCG extract antigens fails in the presence of normal human or rabbit serum or albumin in dilutions to 1:128.

Sumario

Investigaciones Serológicas de los Antígenos Micobacterianos: V. El Influio de los Componentes del Suero Normal sobre la Sensibilización y la Reactividad de los Eritrocitos Ovinos Tratados con Antígeno

Los antígenos de extracto de BCG son separados por lavado de los eritrocitos de oveja por suero normal (de conejo o humano). Se demostró que el factor en la elución estaba vinculado con las fracciones de albúmina. La albúmina bovina posee una propiedad semejante. No se afectan los glóbulos ovinos sensibilizados con tuberculina antigua (TA).

La interacción de los eritrocitos tratados con extracto de BCG y del anticuerpo homólogo impide la separación por lavado (elución) del complejo, del eritrocito al exponerlo después a suero normal.

La sensibilización de los eritrocitos ovinos por antígenos de TA y de extracto de BCG no tiene lugar si hay presentes suero humano o cunicular normal o albúmina a diluciones hasta de 1:128.

Resume

Investigations sérologiques des antigènes mycobactériens: V. L'influence des composants du sérum normal sur la sensibilisation et la réactivité des érythrocytes de mouton traités par un antigène

Les antigènes d'extrait de BCG sont élués à partir de l'érythrocyte de mouton par le sérum normal (lapin ou homme). Le facteur d'élution a été trouvé associé aux fractions

albuminiques. L'albumine bovine possède une propriété similaire. Les cellules de mouton sensibilisées à la tuberculine de Koch (OT) ne sont pas affectées.

L'interaction des érythrocytes traités par l'extrait de BCG et de l'anticorps homologue prévient l'élution du complexe formé à partir de l'érythrocyte lorsqu'il est ensuite exposé au sérum normal.

La sensibilisation des érythrocytes de mouton par les antigènes d'extrait de BCG et de OT fait défaut en présence de sérum normal d'homme ou de lapin ou d'albumine en dilutions à 1/128.

REFERENCES

(1) THURSTON, J. R., RHEINS, M., AND HUZIWARA, T.: Serologic investigations of mycobacterial antigens: I. The specificities of water and saline extracts of BCG, *Mycobacterium phlei*, and H37Rv, Am. Rev. Tubere., 1956, *73*, 563.

(2) THURSTON, J. R., AND RHEINS, M.: Serologic investigations of mycobacterial antigens: II. Specificities of antigens obtained by successive aqueous extraction of BCG and *Mycobacterium phlei*, Am. Rev. Tubere., 1956, *73*, 571.

(3) THURSTON, J. R., AND RHEINS, M.: Serologic investigations of mycobacterial antigens: III. Direct detection of homologous antibodies by BCG extract antigens in serums of rabbits injected with BCG and challenged with *Mycobacterium bovis*, Am. Rev. Tubere., 1956, *74*, 756.

(4) RHEINS, M., AND THURSTON, J. R.: Serologic investigations of mycobacterial antigens: IV. Further serologic characterization of BCG extract antigen, Am. Rev. Tubere., 1956, *74*, 764.

(5) RHEINS, M. S., THURSTON, J. R., NEWTON, N., AND DODD, M. C.: Evaluation of "drop" techniques for detection of hemagglutinins and hemolysins in tuberculous sera, Am. J. M. Technol., 1954, *20*, 254.

(6) THURSTON, J. R., RHEINS, M. S., AND BUEHLER, E. V.: A rapid method for recovering serologically active globulins by Na_2SO_4 precipitation, J. Lab. & Clin. Med., 1957, *49*, 647.

(7) FINE, M., FLOCKS, M., AND FEICHTMEIR, T. V.: Nonspecific hemagglutination inhibiting factor in human serum, Am. J. M. Sc., 1955, *229*, 670.

(8) BOYDEN, S. V., AND ANDERSON, M. E.: Agglutination of normal erythrocytes in mixtures of antibody and antigen, and hemolysis in the presence of complement, Brit. J. Exper. Path., 1955, *36*, 162.

(9) BOYDEN, S. V.: Adsorption by erythrocytes of antigens of *Pfeifferella mallei* and *Pfeifferella whitmori*, Proc. Soc. Exper. Biol. & Med., 1950, *73*, 289.

(10) NETER, E., WESTPHAL, O., AND LUDERITZ, O.: Effects of lecithin, cholesterol, and serum on erythrocyte modification and antibody neutralization by enterobacterial lipopolysaccharides, Proc. Soc. Exper. Biol. & Med., 1955, *88*, 339.

(11) NETER, E., ZALEWSKI, N. J., AND ZAK, D. A.: Inhibition by lecithin and cholesterol of bacterial (*Escherichia coli*) hemagglutination and hemolysis, J. Immunol., 1953, *71*, 145.

(12) BOYDEN, S. V., AND GRABAR, P.: Role des lipides dans la sensibilisation des erythrocytes par les constituents de la tuberculine, Ann. Inst. Pasteur, 1954, *87*, 257.

(13) BOYDEN S. V.: Fixation of bacterial products by erythrocytes *in vivo* and by leucocytes, Nature, 1953, *171*, 402.

FORMATION OF BULLAE OR CYSTLIKE CAVITIES DURING CHEMOTHERAPY IN RABBITS WITH PULMONARY TUBERCULOSIS[1,2]

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INTRODUCTION

The formation of thin-walled bullae during chemotherapy in the lungs of patients with pulmonary tuberculosis has been described in the literature (1–18), and the subject has recently been summarized in a short editorial (19). The lesions have been called giant bullae, emphysematous bullae, bullous cavities, tuberculous cavities with bullous form, cystlike cavities, insufflated cavitation, and thin-walled cavities produced by chemical "casectomy." They have appeared commonly in areas of dense disease during the administration of isoniazid. The so-called open healing of cavities sometimes leads to the formation of similar-appearing large, thin-walled pulmonary air spaces, but these should not be considered in the same light as bullae which appear in positions where no cavity could be demonstrated previously.

In 1952 it was observed in this laboratory that thin-walled bullous cavities frequently appeared early in the course of effective chemotherapy in rabbits infected by inhalation with virulent bovine tubercle bacilli. Reference to this observation was made in 1953 (20). It is the purpose of the present report to present more extensive data on the formation, pathologic anatomy, and ultimate fate of these lesions.

METHODS

Rabbits of mixed breeds and colors, weighing approximately 3.5 kg. each, were forced to inhale a nebulized suspension of *M. tuberculosis* var. *bovis*, strain 4228-4. A two-week culture on solid egg (American Trudeau Society) medium was ground in a mortar, with the gradual addition of sterile physiologic saline. After the clumps had settled, the suspension was diluted with saline until it contained approximately 0.05 mg. dry weight per ml. The rabbit's nose, protruding through a tightly fitted rubber sleeve, was placed in a small glass cylinder, into the other end of which the suspension of bacilli was sprayed by means of a DeVilbiss hand nebulizer. After receiving 5 puffs from the nebulizer, each animal remained in the glass cylinder for thirty seconds.

The progress of disease in the lungs was followed by serial chest roentgenograms, as outlined previously (21). Treatment with various regimens of chemotherapy was instituted when advanced progressive lesions were visible in the roentgenograms, usually three to four months after infection. The regimens, each administered to a group of 8 to 10 rabbits, were as follows: Streptomycin, isoniazid, para-aminosalicylic acid (PAS), streptomycin-PAS, isoniazid-PAS, isoniazid-streptomycin, and isoniazid-streptomycin-PAS. Streptomycin and isoniazid were given intramuscularly once daily, the former in dosage of 150 mg. (43 mg. per kg. per day) and the latter in dosage of 50 mg. (14 mg. per kg. per day).

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[1] From the Trudeau Laboratory, Trudeau Foundation, Inc., Saranac Lake, New York.

[2] This study was aided in part by a research grant (E36 C10) from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, U. S. Public Health Service.

Para-aminosalicylic acid was administered as a 1 per cent solution in the drinking water ad libitum (600 to 800 mg. per kg. per day).

Results

All of the drug regimens used, except PAS alone, were effective in producing a roentgenographic clearing of the lesions. Ten untreated control rabbits showed progressive confluent pneumonic disease which uniformly resulted in death two to seven months after infection. The rabbits treated with PAS alone behaved in similar fashion except that the survival time was slightly prolonged.

The formation of bullae was observed in 33 (61 per cent) of the 54 rabbits treated with effective drug regimens. There was no significant difference in the incidence of bullae formation among the six effective regimens. The initial observation of a cystlike cavity usually was made three to eight weeks after the start of chemotherapy, when a rounded thin-walled radiolucent cavitary lesion suddenly appeared in an area previously occupied by shadows denoting a dense solid lesion. It was not uncommon to see four or five bullae appear simultaneously, although occasionally only a single one was noted. Sometimes the cystlike

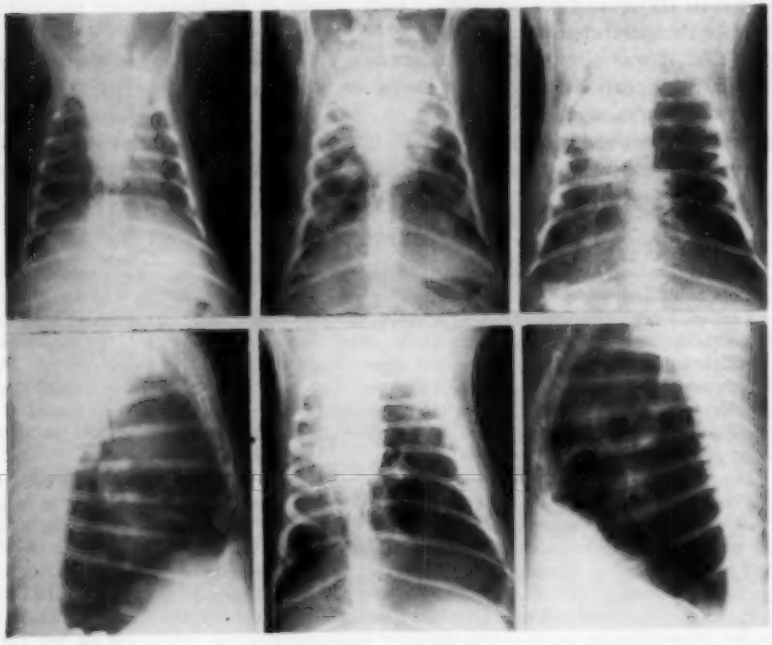

Fig. 1. Rabbit *No. 527*, chest roentgenograms. Upper left: Preinfection. Upper middle: Isoniazid started; many discrete dense lesions. Upper right: After three weeks of therapy; bilateral bullae formation with spontaneous pneumothorax on the left. Lower left: Same as upper right; lateral view. Lower middle and lower right: Two weeks later; at least four bullae visible. The rabbit died one week later.

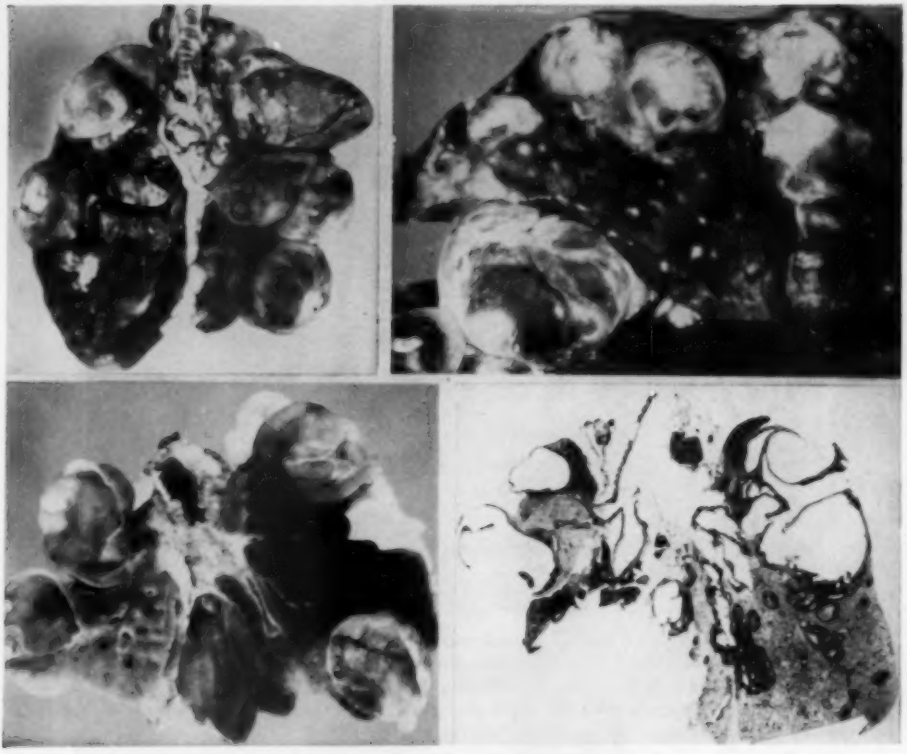

FIG. 2. Rabbit *No. 527*. Upper left and right: Lungs removed at autopsy, showing thin-walled bullae projecting from parenchyma. Lower left: Cut sections through bullae and trachea. Caseous foci and blood vessels may be seen in the walls of the bullae. Lower right: Unmagnified photograph of thin sections through lungs and bullae.

cavities enlarged until they occupied the greater part of the hemithorax, and in several instances rupture of a lesion caused a spontaneous pneumothorax. Both of these circumstances produced shortness of breath and cyanosis.

The case of a rabbit that died of a ruptured lung after six weeks of treatment is illustrated in figures 1 to 3. Many perfectly round thin-walled bullae may be seen in the roentgenogram obtained three weeks after therapy was started. When the lungs were removed at autopsy, it was observed that air forced into the trachea inflated the bullae and the pulmonary parenchyma. Release of pressure on the trachea allowed the lung tissue to deflate, but the air remained trapped in the bullae. In the cut section of the lung (figure 2), a caseous area in the wall of each cyst may be seen projecting into the lumen. The wall itself (*see* figure 3) was extremely thin and translucent and, on the side opposite the caseous focus, consisted only of a few cell layers of compressed alveolar septa without cellular

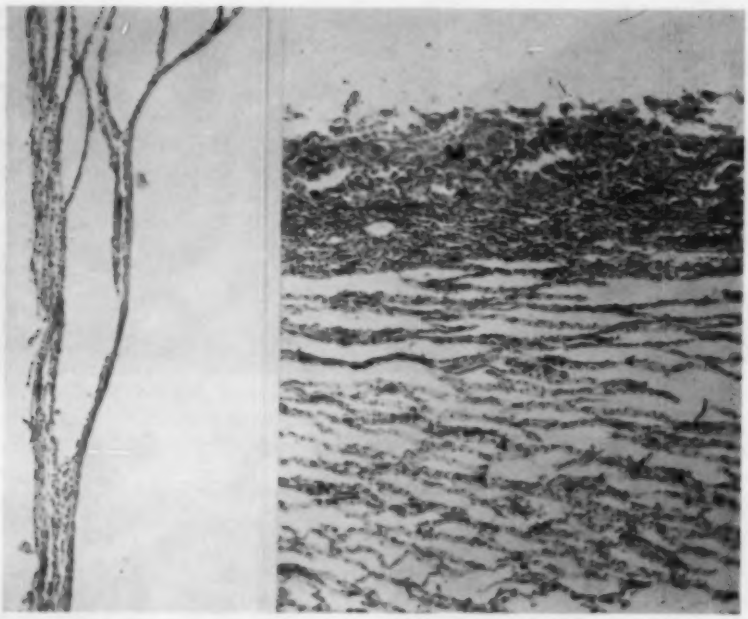

FIG. 3. Rabbit *No. 327*. Left: Photomicrograph through the thin part of the wall of a bulla. It consists of a few cell layers of compressed alveolar septae with slight lymphocytic infiltration. Pleura is at the right. (× 100) Right: Another portion of the bulla, where the wall is made up of caseous material, epithelioid cells, and a thicker peripheral zone of compressed pulmonary tissue infiltrated with lymphocytes. (× 100)

reaction. Elsewhere, the wall was made up of caseous material, epithelioid cells, and a peripheral zone of compressed alveoli infiltrated with lymphocytes.

In rabbits that survived the acute stage, the bullae usually soon lost their rounded contour, shrank in size, and became filled in with caseocalcific material. The lesions were ultimately converted into small calcified scars. Figure 4 illustrates the case of a rabbit that died of causes unrelated to tuberculosis after two hundred and eighty days of isoniazid-streptomycin therapy. A large cystlike cavity in the left lower lobe was converted into a much smaller solid lesion filled with caseocalcific material in which acid-fast bacilli could be seen on microscopy, but from which viable tubercle bacilli could not be recovered by culture or guinea pig inoculation. At autopsy two similar lesions were noted in other areas of the lungs.

The ultimate transformation of the lesion into a calcified scar is illustrated in figures 5 and 6. A cystlike cavity in the right midlung field anteriorly may be followed from the time of its appearance after four weeks of therapy until it presents as a small calcified scar thirteen months after discontinuance of isoniazid-streptomycin. Tubercle bacilli could not be recovered post mortem from

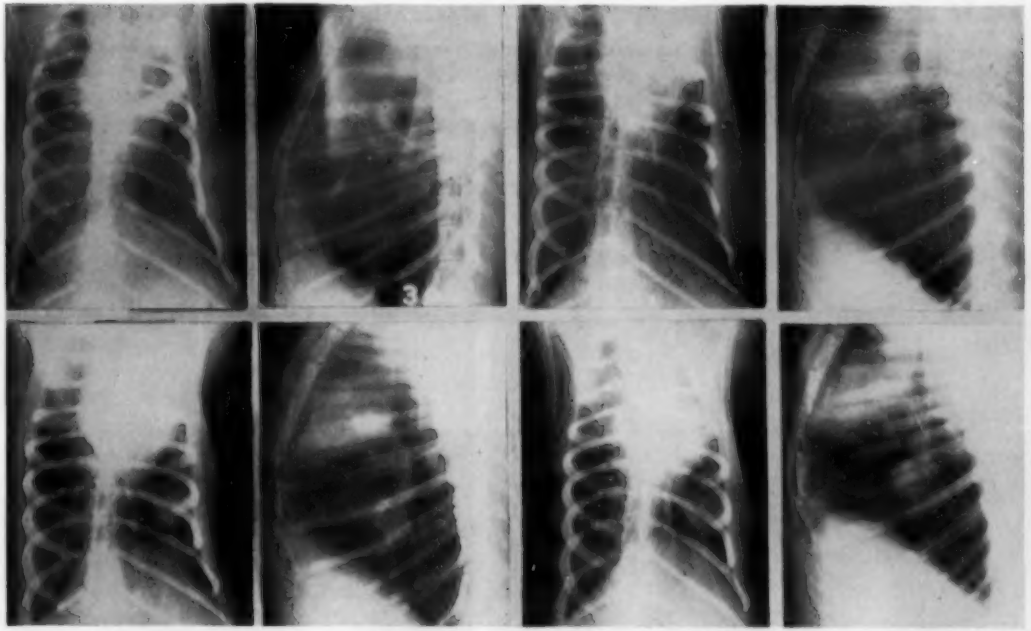

FIG. 4. Rabbit *No. 391*, chest roentgenograms. Upper row, from left to right, 1 and 2: Two large bullae occupying most of left hemithorax, three months after isoniazid-streptomycin was started. 3 and 4: Two months later, the upper bulla is filled in and the lower is much smaller. Lower row, from left to right, 1 and 2: After seven months of treatment, bullae have become solid lesions. 3 and 4: One month later (four weeks before death), lesions are more dense and calcified.

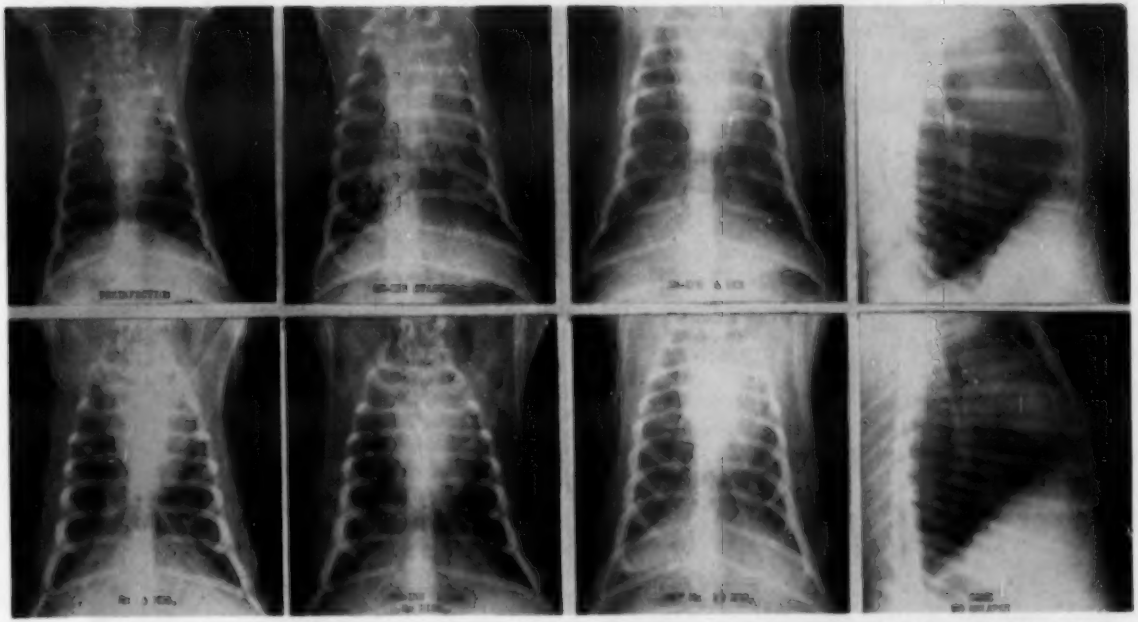

FIG. 5. Rabbit *No. 220*, chest roentgenograms. Upper row: Shows formation of bulla in area of dense disease in the right mid-lung field anteriorly. Lower row: Shows the conversion of the lesion to a solid focus which becomes a small calcified scar after one year of isoniazid-streptomycin followed by thirteen months of observation.

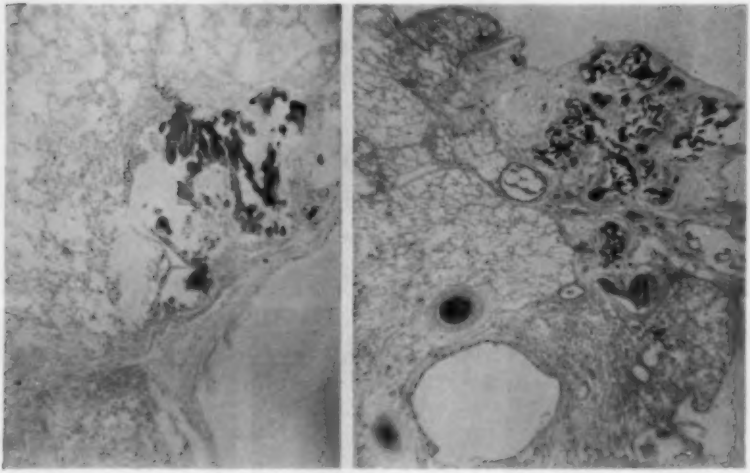

FIG. 6. Rabbit *No. 220*, Photomicrographs of lung. (× 50) Two calcified scars with very little cellular reaction, probably the sites of former bullae.

the calcified lesions, nor were acid-fast bacilli visible on smear. The microscopic appearance of two calcified lesions is shown in figure 6.

Another less common type of healing in which the cystlike cavity suddenly filled with fluid which cast a homogeneous roentgenographic shadow is illustrated by the series of roentgenograms in figure 7.

In a few instances the thin-walled cystlike lesions were converted into irregular thick-walled tuberculous cavities which usually persisted despite chemotherapy and eventually yielded drug-resistant organisms.

DISCUSSION

The mechanism by which the bullous lesions evolved has not been completely established, but it would appear that a combination of two factors was responsible. First, a check-valve type of obstruction must have been produced in the bronchus during the initial stages of healing. It may be postulated that this obstruction was due to inflammatory reaction and edema which later subsided enough to establish open bronchial communication. The continued inflation of the bullae when the lungs were deflated after air had been blown into the trachea was proof of the presence of the check-valve mechanism. Second, a softening of the pneumonic lesions probably occurred during the early stages of chemotherapy, as evidenced by clearing of the roentgenographic shadows just prior to the appearance of the cystlike cavities. The softened material may have been evacuated through the bronchi, thus paving the way for bullae formation.

Although adequate pathologic study of the lesions in the later stages has not yet been made, it did appear grossly at autopsy that most of the filled-in lesions had open bronchial communications.

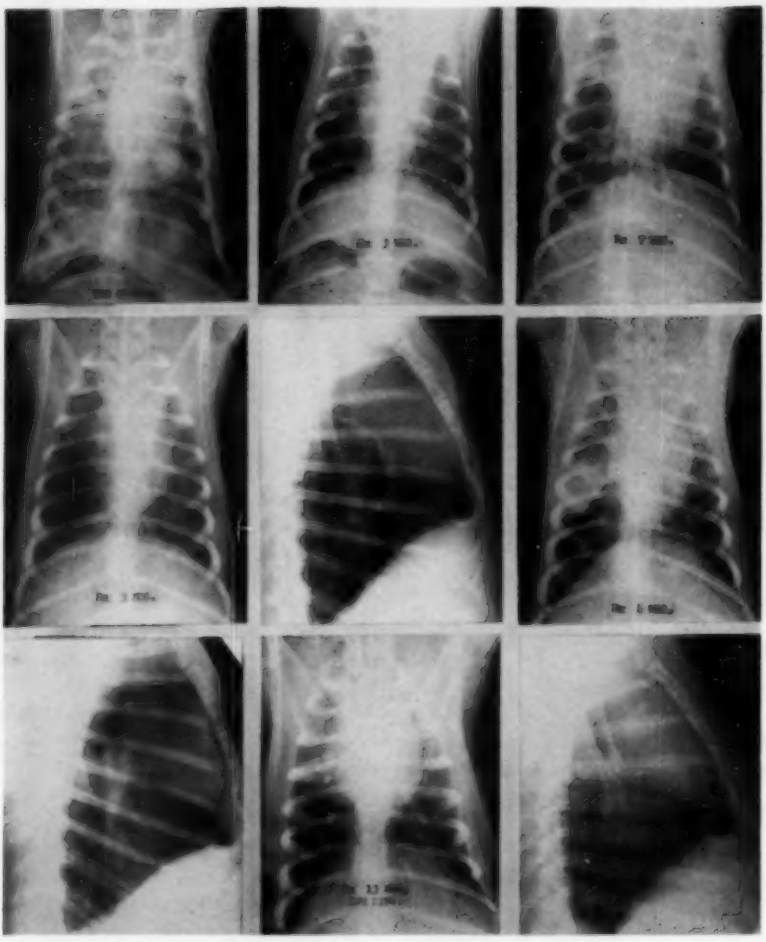

FIG. 7. Rabbit *No. 211*, chest roentgenograms. Upper left: Many discrete and confluent lesions at the time isoniazid was started. Upper middle: Marked clearing of shadows after three weeks of treatment. Upper right: Bulla may be seen in right midlung field. Middle left and center: Bulla has enlarged and extends from third to seventh posterior ribs. Middle right and lower left: One month later, bulla has almost completely filled with fluid. Lower center and right: After thirteen months of isoniazid, lesion is much smaller and does not contain air, but calcification is not prominent.

It should be emphasized that the tissue reactions associated with the early stages of effective chemotherapy were a prerequisite for the formation of bullae. These lesions were not observed in untreated rabbits or in those treated with ineffective drugs, in both of which groups the disease progressed by coalescence of

pneumonic areas. The formation of bullae during effective antituberculous therapy has been noted in rare instances in rabbits infected intravenously, and in another laboratory (22) in rabbits infected by the intratracheal inoculation of virulent bovine tubercle bacilli.

The relationship between the experimentally produced bullous cavities and lesions of similar nature seen in patients cannot be established with certainty from the evidence at hand, but it does not seem too far fetched to postulate that similar mechanisms may be operating in both situations. At any rate, an experimental tool is available for the study of this phenomenon in greater detail.

Summary

The formation of thin-walled bullous cavities has been observed frequently in the lungs of rabbits infected by inhalation of virulent bovine tubercle bacilli. The bullae appeared rather suddenly during the first few weeks of effective chemotherapy in areas previously occupied by dense disease and were not noted in untreated or ineffectively treated animals. Their appearance was related to the formation of check-valve mechanisms in the draining bronchi. During continued chemotherapy, most of the cystlike lesions were converted into solid caseocalcific foci which eventually became small calcified scars.

Sumario

Formación de Flictenas o Cavidades Quistoideas Durante la Quimioterapia en los Conejos con Tuberculosis Pulmonar

En los pulmones de conejos infectados por inhalación de bacilos tuberculosos bovinos virulentos, se ha observado frecuentemente la formación de cavidades vesiculares de paredes delgadas. Las flictenas aparecieron algo repentinamente durante las primeras semanas de quimioterapia eficaz en zonas previamente ocupadas por espesamiento tuberculoso, sin que se observaran en los animales no tratados o tratados ineficazmente. Su aparición se relacionó con la formación de mecanismos de válvula de retención en los bronquios de drenaje. Durante la quimioterapia prolongada, la mayor parte de las lesiones quistoideas se convirtieron en focos cáseo-calcíficos macizos que a la larga se transformaron en pequeñas cicatrices calcificadas.

Resume

Formation de bulles ou d'excavations kystiques durant la chimiothérapie chez des lapins atteints de tuberculose pulmonaire

La formation d'excavations bulleuses à parois minces a été fréquemment observée dans les poumons de lapins inoculés par l'inhalation de bacilles tuberculeux bovins virulents. Les bulles apparaissaient soudainement, au cours des premières semaines d'une chimiothérapie efficace, au niveau de zones antérieurement affectées de lésions denses, et elles faisaient défaut chez les animaux où le traitement se montrait inefficace. Leur apparition est liée à un mécanisme du type clapet dans les bronches de drainage. Au cours de la chimiothérapie, continue, la plupart des lésions kystiques se transforment en foyers caséeux semi-calcifiés denses, qui se sont ultérieurement transformés en lésions cicatricielles calcifiées.

REFERENCES

(1) Jacob, P., Chauveau, J., Cartier, R., and Viveret, J.: Apparition de bulles géantes et régressives au cours du nettoyage par les antibiotiques et en particulier par

l'isoniazide de lésions tuberculeuses diffuses des poumons, Rev. de la tuberc., 1953, *17*, 515.

(2) Bernard, E., and Carraud, J.: Variétés d'aspect et conditions d'apparition d'images bulleuses au cours du traitement de la tuberculose pulmonaire par les antibiotiques, Rev. de la tuberc., 1953, *17*, 1021.

(3) Galy, P., Bérard, M., Arribehaute, P., Touraine, R. G., and de Saint-Florent: Cavernes bulleuses. Documentation anatomo-clinique, Rev. de la tuberc., 1953, *17*, 1037.

(4) Pruvost, P., Delarue, J., Meyer, A., and Depierre: Cavernes tuberculeuses à forme bulleuses, Rev. de la tuberc., 1953, *17*, 1046.

(5) Purriel, P., Casamayou, E., Muras, O., Piovano, C., and Ruggiero, C.: Casectomía química obtenida con la hidracida del ácido isonicotínico (Rimifón) con aprición de defectos mecánicos, alteraciones neumáticas, Tórax (Montevideo), 1953, *2*, 115.

(6) Alarcón, D. G.: Enfisema pulmonar buloso gigante regresivo en la tuberculosis del adulto, Rev. mex. tuberc., 1954, *15*, 399.

(7) Jacob, P., Cartier, R., and Treps, P.: Quelques recherches tendant a préciser la cause et la nature des images bullaires observées au cours de la régression thérapeutique de tuberculoses nodulaires diffuses, Rev. de la tuberc., 1954, *18*, 85.

(8) Chenebault, J.: Étude anatomo-radiologique des cavernes pulmonaires tuberculeuses détergées et distendues, de type bulleux, Rev. de la tuberc., 1954, *18*, 189.

(9) Bernou, A., and Brun, J.: Cavernes détergées et néo-cavités bulleuses après traitement chimiothérapique, Presse méd., 1955, *63*, 894.

(10) Brun, J., Guichard, A., and Combey, P.: Emphysème bulleux et néocavités de substitution après rétraction des ulcerations tuberculeuses, Rev. Lyon. méd., 1955, *4*, 143.

(11) Barrie, J., and Roulet, A.: Évolution vers l'aspect polykystique des lésions d'un ensemencement bronchogène sous l'influence d'un traitement par l'isoniazide, Rev. de la tuberc., 1955, *19*, 91.

(12) Martinez, J. A., and Cassassas, O.: Insufflated pulmonary tuberculous cavitation in infants, Med. clín. (Barcelona), 1955, *24*, 352.

(13) Calciu, M., and Grunberg, I.: Bullous tuberculosis, Ftiziol. (Bucharest), 1955, *4*, 28.

(14) Sartori, C.: Immagini bollose in corso di tubercolosi pulmonare trattatà con chemioantibiotici, Riv. pat. e clin. tuberc., 1955, *28*, 88.

(15) Johnsen, L., and Hewitt, W. C.: Cystlike cavities with isoniazid therapy in pulmonary tuberculosis, Am. Rev. Tuberc., 1954, *69*, 1054.

(16) Rappaport, I.: Chemotherapy and "vanishing lungs," J. A. M. A., 1955, *158*, 1436.

(17) Russell, W. F., Jr., Dressler, S. H., Middlebrook, G., and Denst, J.: Implications of the phenomenon of open cavity healing for the chemotherapy of pulmonary tuberculosis (editorial), Am. Rev. Tuberc., 1955, *71*, 441.

(18) Shek, J. L., Cope, J. A., and Myers, G. D.: Giant air cyst(s) as a sequela of pulmonary tuberculosis, J. Thoracic Surg., 1956, *32*, 96.

(19) Berthrong, M.: Pulmonary "cyst" formation following treatment of tuberculosis with antibiotics, Am. J. Clin. Path., 1956, *26*, 396.

(20) Steeken, W., Jr., Wolinsky, E., Pratt, P. S., and Costigan, W. J.: Effect of antibacterial agents on chronic pulmonary tuberculosis in rabbits: A roentgenographic, pathologic, and bacteriologic study, Nat. Tuberc. A. Tr., 1953, p. 218.

(21) Steenken, W., Jr., Wolinsky, E., Bristol, L. J., and Costigan, W. J.: Use of the rabbit in experimental tuberculosis: I. A visual method of evaluation of antituberculous agents by serial chest roentgenograms, Am. Rev. Tuberc., 1953, *68*, 65.

(22) Kazlowski, J., Raleigh, J. W., and Steenken, W., Jr.: Unpublished data.

REMARKS ON THE EPIDEMIOLOGY OF TUBERCULOSIS[1]

A Review

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Introduction

A phenomenon of greatest importance to those interested in the control of tuberculosis is the extent to which this disease now affects older people. As mentioned by Dempsey (1), "It is difficult to realize that today half of all deaths from tuberculosis in this country are those of men 40 years of age and older." Robins (2) points out that "The most significant trend in the epidemiology of tuberculosis is the degree to which the disease is affecting older people, particularly older men." The beginning of this trend was noted nearly twenty years ago by Drolet (3); the trend has been remarked upon more recently by numerous workers (4–10).

The cause for the shift in tuberculosis mortality to the older age group remains undetermined. It is still a moot question whether ". . . it is erroneous to consider tuberculosis in this age group as a common vestige from previous decades" (9) or whether one should accept the considerable amount of evidence that ". . . the high mortality of old age is the residual of the tuberculosis through which the older groups have passed . . ." (10).

Actually there are three facets of the tuberculosis problem which are of particular interest to epidemiologists: (*1*) the shift in maximal mortality to older age groups, (*2*) the reversal in the sex ratio of deaths from tuberculosis, and (*3*) the seeming paradox, in view of item (*1*) above, that the maximal hazard of death from tuberculosis for a group of adults born at about the same time has shifted to a *younger* age group than was the case thirty years ago. It is possible that a common factor or factors may be of importance in explaining all three of these items.

Observations

Tuberculosis Mortality Data

The mortality from tuberculosis in different age groups in successive decades is shown in table 1. Plotting vertical columns from table 1 gives age-specific mortality curves which show the experience of various age groups in one given year (figure 1). It will be noted that the curves for 1900 and again for 1920 show a peak in the 25- to 34-year age groups. By 1950 this peak had disappeared, the curve showing instead a gradual and fairly consistent increase in mortality from the 5- to 14-year age group to a maximum in the oldest group. From the shape of the curve it is evident that in the year 1950 there were more tuber-

[1] From the Wm. H. Maybury Sanatorium (Detroit Municipal Tuberculosis Sanatorium), Northville, Michigan.

TABLE 1

AGE-SPECIFIC MORTALITY FROM TUBERCULOSIS IN SUCCESSIVE DECADES
(Death rate per 100,000 population in given age group)

Age Group	1880*	1890*	1900†	1910†	1920†	1930†	1940†	1950‡
0–4			143.8	110.3	57.6	31.0	14.8	6.7
5–14			36.2	29.7	22.4	11.9	5.5	1.8
15–24	278.7	242.7	205.7	152.0	136.1	77.3	38.2	11.3
25–34	361.5	340.1	294.3	217.6	164.9	102.8	56.3	19.1
35–44	314.2	305.7	253.6	214.9	147.4	92.4	59.4	26.1
45–54	279.0	266.8	215.6	188.1	137.2	93.2	66.3	35.9
55–64	347.5	288.7	223.0	192.9	141.3	97.0	76.1	47.7
65–74	498.4	349.9	256.1	203.5	163.8	111.7	80.8	57.7
75–84	639.5	365.4	279.3	189.2	157.7	115.5	80.4	63.2

* Mortality from *pulmonary* tuberculosis. Data from article by Putnam (11). Death rates for all forms of tuberculosis would be slightly higher.

† Mortality from tuberculosis (all forms). Data from Linder and Grove (12). Values for age group 0–4 estimated from values given for age groups 0–1 and 1–4.

‡ Mortality from tuberculosis (all forms). Data from U. S. Public Health Service Vital Statistics (13).

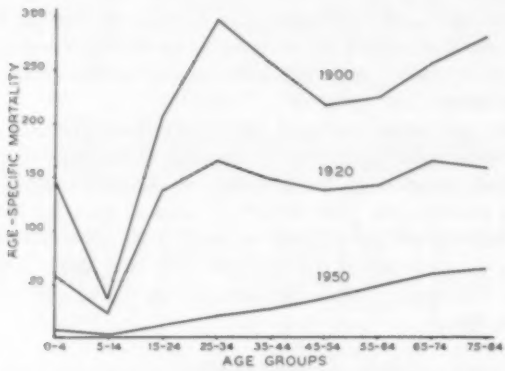

FIG. 1. Age-specific tuberculosis mortality curves for 1900, 1920, and 1950.

culosis deaths per 100,000 persons in the 75- to 84-year group than in any other. However, the important point to remember is that at some time in their history the members of this 75- to 84-year group, and all of the other older age groups, experienced a *higher* mortality from tuberculosis than in 1950, a fact clearly shown in "generation" curves.

Mortality Curves for Generations

In table 1 the age groups are given in 10-year intervals and the mortality data are given by decades. Frost (14) has pointed out that, if data from such a table are plotted diagonally from upper left to lower right, a picture is presented of

the tuberculosis mortality experienced by the survivors in each decade of a group of individuals born at about the same time. Such a group has been called by Frost a "cohort." Possibly the term "generation," suggested by Lancaster (15), is more appropriate, even though ten years is considerably shorter than the period of time usually allotted to a generation. Whatever their name, curves of this type give information which is essential to an understanding of the epidemiology of tuberculosis, for "In a chronic disease such as tuberculosis it is difficult to understand the mortality rates of today without considering what has happened to a given age group in preceding decades" (14).

A series of generation curves is presented in figure 2. Because of incomplete data in table 1 only the five lowest curves could be drawn to include the 1- to 4-year age group. The four generations represented in curves *a*, *b*, *c*, and *d* were born about 1860, 1870, 1880, and 1890, respectively. It will be seen that joining the final (1950) reference points of the curves in figure 2 would give the bottom curve in figure 1.

As indicated in curves *e*, *f*, and *g*, the highest tuberculosis mortality for each generation represented was experienced in the youngest group, a secondary mortality peak being reached when the generation reached the age of 20 plus-minus 5 years. In contrast to this, the generations represented by curves *a*, *b*, *c*, and *d* experienced their secondary peak of tuberculosis mortality a decade later at the age of 30 plus-minus 5 years. In these four uppermost curves a fairly consistent parallel will be noted, the secondary peak appearing quite definitely in the 25- to 34-year age group. In 1920, when data were available for only these four curves, one might have assumed that the age of 30 plus-minus 5

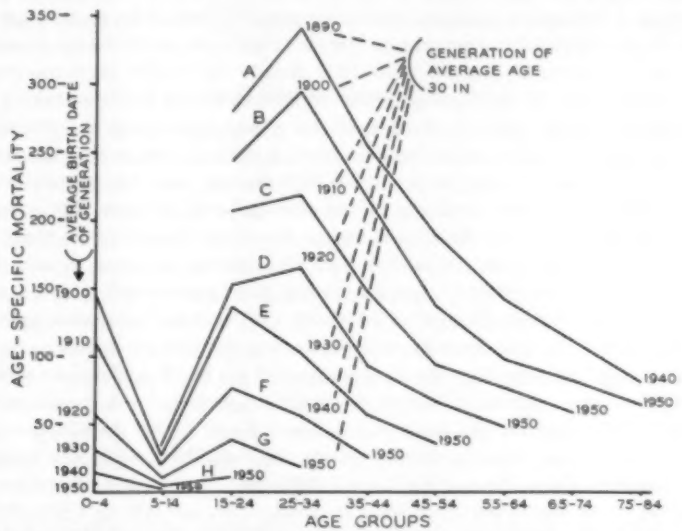

FIG. 2. Age-specific mortality from tuberculosis of various generations in the United States.

years was the period of greatest tuberculosis hazard for adults in any generation. However, beginning with the next generation, that born about 1900, the secondary mortality peak is found at the age of 20 plus-minus 5 years and it has remained at that point since. A similar shift in the tuberculosis mortality peak to a younger age group has been observed in other countries, i.e., by Lancaster (15) in Australia and by Springett (16) in England and Wales.

The Shift of Maximal Adult Mortality to a Younger Age Group

One reason for the occurrence of the peak of adult tuberculosis mortality in the 25- to 34-year age group during the early years of the present century is the fact that persons infected with tubercle bacilli are gradually accumulated as a given generation increases in age (17). However, if this were the main reason, one would expect the peak to occur in an older group than that, aged 30 plus-minus 5 years. It seems to the present writer that a more important reason for this peak of adult tuberculosis mortality, occurring as it did in each decade up to and including 1920, is the fact that persons in the 25- to 34-year age group have been the chief breadwinners and homemakers in each generation and, in that capacity, have been chiefly subject to the heavy physical demands of industry, the home, and the farm. Terris (18) has suggested that physical overstrain plays a significant role in the development of active tuberculosis, and Burke (19) has demonstrated that periods of exercise have a deleterious effect on guinea pigs infected with tubercle bacilli. Selye (20) also has indicated the importance of *stress* in the causation of various diseases including tuberculosis.

If one admits that physical overstrain may have been an important factor in causing the peak of tuberculosis mortality in the 25- to 34-year age group, then any marked change in working conditions may be looked to as a cause for a change in the shape of the generation curves. That some such change in working conditions did occur during the 1920–1930 decade can readily be demonstrated.

The substitution of mechanical power for human brawn has been taking place gradually ever since the introduction of the steam engine, but the process was markedly accelerated with the exploitation of electric power and the internal combustion engine. It was in the 1920–1930 decade that the automobile was popularized and became available to the average working man. Total registration of motor vehicles in the United States rose from slightly more than 9 million in 1920 to more than 26 million in 1930. During the same decade the installed capacity of electric utility generating plants increased from 13 million kilowatts to more than 32 million kilowatts (21) and the increased generating capacity flowed into thousands of labor-saving devices in industry. In 1930 cement was being mixed by machine instead of by hand, and bricks were carried to the upper stories of a new construction project by a freight elevator instead of in a hod on the back of a laborer. During this decade the farmer began to have more leisure, evidenced by the fact that, while the horse and mule population was decreasing by 6.5 millions, the number of tractors and trucks on farms increased more than fourfold, from 400,000 to 1,800,000 (22). In the home, vacuum cleaners began to replace the household broom, and

power washing machines the old fashioned tub and washboard. Since the 25- to 34-year age group comprises the largest labor pool in each 10-year period, this group might be expected to benefit first from the changes detailed above.

One other factor should be mentioned. The fifteen years prior to 1930 saw an upsurge in social consciousness, with the construction of tuberculosis sanatoriums on a large scale. In the state of Michigan, for example, there were only 573 beds for tuberculous patients in 1915. By 1920 this number had increased to 1,094. In 1928 there were 2,710 beds and by 1930 the number of beds for tuberculous patients had increased to 3,722 (23). This increased bed capacity, with the opportunity it gave for isolating cases of open tuberculosis, was of great significance from the point of view of preventing infection in the home.[2] Probably of equal significance from the point of view of cutting down tuberculosis mortality was the relief afforded the overworked housewife who had been forced to care for a tuberculous husband or child.

Improved working conditions and the opportunity to isolate cases of "open" tuberculosis in sanatoriums have been mentioned as important reasons for the marked decrease in tuberculosis mortality which occurred, particularly in the 25- to 34-year age group during the 1920–1930 decade. However, the question remains, why shouldn't the 15- to 24-year group have benefited equally? As indicated in figure 2, the maximal adult mortality from tuberculosis shifted to this younger group during the 1920–1930 decade and has remained there since. Springett (24), in an attempt to explain this shift, suggests that "A selective arrest in the decline of mortality in young adults . . . took place in the 1920's–1930's because of a substantial transference of primary infection from childhood to this age group." Perhaps of more significance is the preponderance of female over male deaths which has occurred for many decades in the 15- to 24-year age group.

Tuberculosis Mortality According to Age and Sex

During most of the nineteenth century the over-all death rate from tuberculosis among females in the United States was considerably higher than among males. In Massachusetts, for example, the female death rate in 1850 was 40 per cent more than that in males (10). Operatives in the cotton mills of Fall River, Manchester, and Pawtucket, even as late as 1907, had a tuberculosis death rate varying from 219 per 100,000 in young women 20 to 25 years of age to a maximum of 473 in women 30 to 35 years of age (25). In Massachusetts today the mortality for the male sex from tuberculosis is 2.8 times that for females (10).

In table 2, tuberculosis death rates for the entire United States are given for both sexes beginning with the 15-year group. Also the *ratio* of male to female death rates is indicated. A glance at the table will show that only in the 15- to 24-year age group is the female mortality significantly higher than the male mortality, with a male:female ratio of 0.65 in 1940 and 0.66 in 1950. Why should this be? Israel and DeLien (26), after observing a group of tuberculin-positive

[2] In the entire United States the proportion of tuberculous patients "isolated" increased from 4 per cent in 1915 to 25 per cent in 1934 (3).

children through adolescence and early adult life, found clinical tuberculosis developing more than twice as frequently in white females as in white males and more than three times as frequently in Negro females as in Negro males. Likewise, Lincoln (27), in a long-term follow-up observation of children with primary tuberculosis, found that chronic pulmonary tuberculosis developed more than twice as frequently in females as in males and that in most girls the first roentgenographic abnormality was found within two years of menarche. As suggested by Ryan many years ago (28), "The onset of puberty is especially dangerous to the girl with an active or quiescent pulmonary tuberculosis." Thus it seems evident that the biologic changes of adolescence, particularly in the female sex, are highly important in determining the development of active tuberculosis from quiescent disease.

The changes occurring during adolescence have always played a role in tuberculosis mortality (29). Moreover, it is possible that the very family car which has helped to ease the workday of the head of the family has tended to encourage "the exuberance of youth" of the teen-age group. It seems likely, then, that biologic factors not affected by improved working conditions are responsible for the fact that tuberculosis mortality in the 15- to 24-year age group has declined at a slower rate than mortality in the next older group.

As shown in table 2, the death rates for the two sexes in the 25- to 34-year group were almost exactly equal in 1940 and 1950, as indicated by the ratios 1.0 and 0.96. The excess of male over female deaths begins with the 35- to 44-year group and, in 1950, reaches the surprising ratio of 4.1 in the 55- to 64-year age group (figure 3). This very high ratio may be explained in part by the accumulation of men with tuberculosis who might have died in young adult life had it not been for the improvement in working conditions of the 1920–1930 decade. There is the additional factor that an unusual exposure to tuberculosis

TABLE 2

TUBERCULOSIS MORTALITY ACCORDING TO AGE AND SEX

Age Group	Sex	1940		1950	
		Mortality	Ratio	Mortality	Ratio
15–24	Male	30.1		9.0	
	Female	46.1	0.65	13.6	0.66
25–34	Male	56.3		18.7	
	Female	56.3	1.0	19.5	0.96
35–44	Male	74.8		33.5	
	Female	44.0	1.7	18.9	1.8
45–54	Male	95.6		55.7	
	Female	35.3	2.7	16.2	3.5
55–64	Male	110.0		76.7	
	Female	40.6	2.7	18.7	4.1
65–74	Male	106.3		89.9	
	Female	55.7	1.9	27.8	3.2
75–84	Male	93.3		88.7	
	Female	68.7	1.4	41.5	2.1

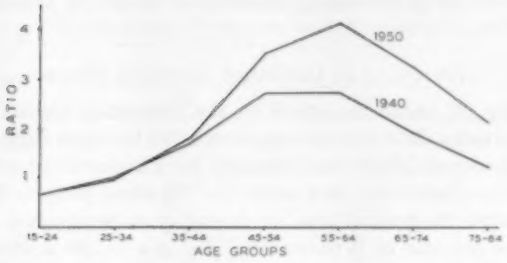

FIG. 3. Age-specific male:female tuberculosis mortality ratios in 1940 and 1950.

was experienced by this generation during war time. In 1917, when the United States entered the first World War, men of the 60-year-old generation of 1950 were then aged 27 plus-minus 5 years. This is the generation which bore the brunt of the fighting in Germany and of exposure to the tuberculosis which was rampant in Europe during and after the war. Men of the next younger generation also participated in the exposure to tuberculosis not only in Europe, but in the cantonments in this country.[3]

The unfavorable conditions under which older, homeless men live have been mentioned by Jones and Roberts (31) as important tuberculogenic factors. Such conditions undoubtedly contribute to the development of active tuberculosis from a quiescent lesion but, if living conditions were the most important factor, one would expect a progressive increase with age in the male:female mortality ratio instead of the abrupt peak shown in figure 3.

Many factors are involved in the complete reversal in the sex ratio of mortality from tuberculosis which has occurred during the past century. First in chronologic sequence, perhaps, was the gradual spread of knowledge concerning the infectious nature of the disease. This was followed by the construction of tuberculosis sanatoriums which afforded an opportunity for isolating cases of open tuberculosis—certainly of great benefit to members of the female sex who were often saddled with the responsibility of caring for patients in the home.

Of importance also was the campaign against bovine tuberculosis initiated by the Bureau of Animal Industry in 1917 (32) and successfully completed during the 1920's and 1930's. Again, the mothers and their daughters, who had had to care for infants infected with tubercle bacilli, were particularly benefited. The better control of diabetes with insulin has helped to decrease tuberculosis mortality, particularly in women of more than 40, who are more subject to diabetes than are men in that age group. And it is probable that conditions of labor in industry have shown greater improvement relatively for women than for men. Certainly no working conditions could have been much *worse* than those in the cotton mills of New England early in the century. Finally, the World War I

[3] The extent of the tuberculosis problem resulting from World War I is indicated by the fact that in 1922 there were more than 44,000 veterans hospitalized for tuberculosis (30).

had a selective effect in increasing tuberculosis mortality among men of the United States (30).

Aberrations in Generation Mortality Curves

In 1952 Spicer (7) began an article on the generation method of analyzing tuberculosis mortality data with the statement "It has been suggested by Frost (1939) and by Andvord (1930) that mortality from tuberculosis can be explained on the assumption that every generation has the same pattern of mortality at various ages, while its general level of mortality is determined very early in life and remains peculiar to it thereafter." Using a simple arithmetic method, Spicer proceeded to test this hypothesis by calculating for each generation what the mortality from respiratory tuberculosis should be at various ages, the generation having started with a certain mortality when 5 to 14 years of age. Comparison of the observed with the calculated rates from 1856 until 1930 showed them to be in very good agreement. In the period from 1930 to 1950, however, there were significant discrepancies. Spicer noted a sharp peak of mortality among males at 55 to 65 years of age not predicted by the generation hypothesis. Female mortality in the middle age groups was greatly overestimated and the mortality rate in youths, both male and female, was underestimated. Spicer concluded "... that these discrepancies cannot be due to chance and that the generation effect has become obscured by other factors." This conclusion would seem to be amply justified. Factors which may have obscured "the generation effect" are the accumulation of potentially tuberculous males of the average age of 60 because of unusual exposure during World War I and because improved working conditions have spared many who, under less favorable conditions, would have died from tuberculosis in early adult life; improved conditions in industry and in the home which have benefited women in the middle age groups particularly; and biologic factors not susceptible to improved working conditions which weigh heavily against the 15- to 24-year age group.

In a sense, all of the 1950 values given in table 1 could be called aberrations, since they have been altered by unexpected influences. These values represent in part the summation of two opposing factors: (*1*) the effect of war with its hardships and exposure of many young adults to pandemic tuberculosis in foreign lands; and (*2*) the ameliorating effect of antimicrobial therapy. The increased tuberculosis mortality resulting from World War II and the Korean War should be seen particularly in age groups 15 to 24 and 25 to 34. One might expect a definite rise at these points in the age-specific tuberculosis mortality curve for 1950 were it not for antimicrobial therapy. Antimicrobial therapy has benefited all age groups, but particularly those younger persons in whom acute exudative disease commonly occurs (table 3).

Instead of a rise over the young adult groups, the 1950 curve (in figure 1) shows tuberculosis mortality gradually increasing with age, as has already been mentioned. The deaths in these older age groups are believed to represent in large part the breakdown of tuberculous disease which the individuals have

TABLE 3

AGE-SPECIFIC MORTALITY FROM TUBERCULOSIS

Age Group	Year		Per Cent Decrease
	1940	1950	
0–4	14.8	6.7	55
5–14	5.5	1.8	67
15–24	38.2	11.3	70
25–34	56.3	19.1	66
35–44	59.4	26.1	56
45–54	66.3	35.9	46
55–64	76.1	47.7	37
65–74	80.8	57.7	29
75–84	80.4	63.2	21

carried many years for, as pointed out by Springett (16), "Despite the present form of the tuberculosis mortality curve, there has never been a group of men who experienced a greater tuberculosis mortality at ages over 45 than they had experienced at young adult ages. The clinical counterpart of this statement is that tuberculosis deaths in later life are usually, though not always, the result of disease acquired many years earlier. They are comparatively seldom the result of recently acquired disease."

Actually the point of view expressed by Springett has gained wide acceptance (10, 33–35). One implication of this point of view might be that every young man who on entering the armed services had a negative tuberculin test and who on discharge had a positive test should be entitled to claim service-connected disability in case of a tuberculous "break" in later life. This would mean a considerable increase over the 15,000 reported in 1950 (36) in the number of tuberculous veterans hospitalized at federal expense.

The importance which Frost (14) originally attached to generation (or "cohort") mortality curves was predicated upon the almost universal infection with tubercle bacilli, which, during the nineteenth century, occurred in infants and young people (37). Corresponding to this high infection rate, generation mortality curves in the past have been characterized by an infant tuberculosis death rate higher than any adult rates in the same generation (curves *e*, *f*, *g*, and *h* in figure 2). The curve for the generation born about 1950 has started with an infant mortality of only 6.7. The extension of this curve will obviously be very flat if the generation never experiences a death rate greater than 6.7. What are the chances that this will be true? In favor of a continued low tuberculosis death rate in the oncoming generation is the fact that so much effort is being expended in this country in an attempt to find and hospitalize every adult with "open" tuberculosis. Against the continued low death rate is the fact that Americans are becoming increasingly prone to travel widely in various parts of the world where infectious tuberculosis is more prevalent. The future course of the mortality curves of our younger generations may then be determined largely by the extent of their exposure to tuberculosis in foreign lands. Until

tuberculosis control is world-wide in scope it is possible that today's youthful generations in America may have a higher tuberculosis mortality as adults than they experienced as infants or young children.

Summary

Although the tuberculosis mortality curve for 1950 shows that death rates are highest in the 75- to 84-year age group it is important to realize that this and the other adult groups have experienced much higher death rates in previous decades. In 1930 the peak of adult tuberculosis mortality (for a given generation) shifted from the 25- to 34-year to the next younger (15- to 24-year) age group. The markedly lessened mortality of the 25- to 34-year group is believed to be due, chiefly, to improved working conditions experienced by this group since 1920. Mortality in the next younger group has not been affected to a similar degree presumably because of biologic factors peculiar to that age group.

Suggestions are made concerning factors responsible for the complete reversal in the sex ratio of mortality from tuberculosis which has occurred during the past century.

Sumario

Observaciones sobre la Epidemiología de la Tuberculosis. Revista

Aunque la curva de la mortalidad tuberculosa para 1950 muestra que los coeficientes de mortalidad alcanzan su máximo en el grupo de 75 a 84 años de edad, es importante comprender que este y otros grupos de adultos han experimentado coeficientes de mortalidad mucho más altos en decenios anteriores. En 1930, la cúspide de la mortalidad tuberculosa en adultos (para una generación dada) se desvió del grupo de 25 a 34 años de edad al grupo más joven siguiente (15 a 24 años). La mortalidad decididamente menor del grupo de 25 a 34 años de edad parece deberse, principalmente, al mejoramiento de las condiciones de trabajo de ese grupo desde 1920. La mortalidad del grupo más joven siguiente no se ha visto afectada en grado semjante, presuntamente debido a factores biológicos peculiares de ese grupo.

Se ofrecen sugerencias acerca de los factores causantes de la inversión total de la proporción por sexos de la mortalidad tuberculosa, que ha tenido lugar durante el siglo pasado.

Resume

Remarques sur l'épidémiologie de la tuberculose—Une revue

Bien que la courbe de mortalité de la tuberculose pour 1950 montre que les taux de mortalité sont les plus élevés dans le groupe de 75 à 84 ans, il est important de réaliser que ce groupe et les autres groupes d'adultes présentaient des taux de mortalité beaucoup plus élevés dans les trente années précédentes. En 1930, le taux maximum de mortalité chez l'adulte (pour une génération donnée) passait du groupe d'âge de 25 à 34 ans au groupe suivant, le groupe de 15 à 24 ans. La mortalité nettement abaissée dans ce groupe de 25 à 34 ans est considérée comme principalement due à l'amélioration des conditions de travail du groupe à partir de 1920. La mortalité du groupe suivant, plus jeune, n'a pas été affectée à un degré similaire, probablement en raison des facteurs biologiques particuliers influant sur ce groupe.

L'auteur formule des hypothèses concernant les facteurs responsables du renversement complet du taux de mortalité par tuberculose, à l'égard du sexe, qui s'est produit au cours du siècle dernier.

REFERENCES

(1) Dempsey, M.: Trends in tuberculosis, N. T. A. Bull., 1954, p. 7.

(2) Robins, A. B.: The age relationship of cases of pulmonary tuberculosis and their associates, Am. J. Pub. Health, 1953, *43*, 718.

(3) Drolet, G. J.: Present trend in case fatality rates in tuberculosis, Am. Rev. Tuberc., 1938, *37*, 125.

(4) Pinner, M.: Pulmonary Tuberculosis in the Adult, ed. 1, Charles C Thomas, Springfield, Illinois, 1945.

(5) Smith, D. T.: Tuberculosis today and tomorrow, Am. Rev. Tuberc., 1953, *67*, 707.

(6) McDonald, J. C., and Springett, V. H.: The decline of tuberculosis mortality in Western Europe, Brit. M. Bull., 1954, *10*, 77.

(7) Spicer, C. C.: The generation method of analysis applied to mortality from tuberculosis, J. Hyg., 1954, *52*, 361.

(8) Anderson, R. J.: Tuberculosis morbidity and mortality: The current trends, paper presented at the 51st annual meeting of the National Tuberculosis Association, May, 1955.

(9) Medlar, E. M.: The behavior of pulmonary tuberculous lesions, Am. Rev. Tuberc., 1955, *71* (Supplement, p. 235).

(10) Pope, A. S., and Gordon, J. E.: The impact of tuberculosis on human populations, Am. J. M. Sc., 1955, *230*, 317.

(11) Putnam, P.: Sex differences in pulmonary tuberculosis deaths, Am. J. Hyg., 1927, *7*, 663.

(12) Linder, F. E., and Grove, R. D.: Vital Statistic Rates in the U. S. 1900-1940, U. S. Gov. Printing Office, 1943.

(13) U. S. Public Health Service Vital Statistics, Special Reports, 1950, *37*, 308.

(14) Frost, W. H.: The age selection of mortality from tuberculosis in successive decades, Am. J. Hyg., 1939, *30*, 91.

(15) Lancaster, H. O.: Tuberculosis mortality in Australia, M. J. Australia, 1950, *1*, 655.

(16) Springett, V. H.: An interpretation of statistical trends in tuberculosis, Lancet, 1952, *1*, 521.

(17) Bentley, F. J., Grzybowski, S., and Benjamin, B.: Tuberculosis in Childhood and Adolescence, National Association for the Prevention of Tuberculosis, London, 1954.

(18) Terris, M.: Relation of economic status to tuberculosis mortality by age and sex, Am. J. Pub. Health, 1948, *38*, 1061.

(19) Burke, H. E.: Some observations concerning the pathogenesis of tuberculosis, paper presented at the 51st annual meeting of the National Tuberculosis Association, May, 1955.

(20) Selye, H.: Stress and disease, Science, 1955, *122*, 625.

(21) U. S. Department of Commerce, Bureau of the Census, Statistical Abstracts of the U. S. Govt. Printing Office, Washington, D. C., 1954.

(22) Fowler, C. B., Griffin, J. I., Cohen, J. B., Greenwald, W. I., Cropsey, J., and Sethur, F.: Economic Handbook, Thomas Y. Crowell, New York, 1955.

(23) Data from the library of the Michigan Tuberculosis Association, through the kindness of T. J. Werle.

(24) Springett, V. H.: An interpretation of statistical trends in tuberculosis, Lancet, 1952, *1*, 575.

(25) Drolet, G. J., and Lowell, A. M.: Whereto tuberculosis?, Am. Rev. Tuberc., 1955, *72*, 419.

(26) Israel, H. L., and DeLien, H.: The relation of childhood infection to the development of tuberculosis in early adult life, Am. J. Pub. Health, 1942, *32*, 1146.

(27) Lincoln, E. M.: The value of follow-up studies of children with primary tuberculosis, Am. Rev. Tuberc., 1951, *64*, 499.

(28) Ryan, C. A.: Studies on tuberculosis in children, J. Mich. M. Soc., 1929, *28*, 307.
(29) Miller, J. A.: Changing scenes in tuberculosis, Am. Rev. Tuberc., 1942, *45*, 637.
(30) Long, E. R.: The war and tuberculosis, Am. Rev. Tuberc., 1942, *45*, 616.
(31) Jones, H. W., and Roberts, J.: Incidence of tuberculosis among homeless men, J. A. M. A., 1954, *155*, 1222.
(32) Myers, J. A.: 30,000 per 100,000, Dis. of Chest, 1956, *30*, 1.
(33) Dubos, R.: Biologic and epidemiologic aspects of tuberculosis, Am. Rev. Tuberc., 1953, *68*, 1.
(34) Perkins, J. E.: Editorial: Our principal problem, N. T. A. Bull., 1956, p. 2.
(35) Willis, H. S.: The President's Column, N. T. A. Bull., 1956, p. 31.
(36) Barnwell, J. B.: Care of the tuberculous veteran, a continuing problem, J. A. M. A. 1951, *146*, 1372.
(37) Long, E. R.: The people against tuberculosis in medicine, Nat. Tuberc. A. Tr., 1954, p. 21.

Notes

TRIALS WITH TUBERCULOSIS VACCINE, IRRADIATED

Treatment with the newer drugs has reduced considerably the mortality rate from tuberculosis during recent years. However, this reduction in mortality has not been accompanied by a proportional reduction in the incidence of tuberculous infection, and the world morbidity remains high. Various attempts have been made to reduce the incidence of tuberculosis by increasing the resistance through vaccination. In most cases the vaccines were prepared from viable attenuated strains such as the murine vole bacillus, the BCG of bovine origin, and the R_1 strain of human origin of Saranac Lake. In addition, a few trials have been made with killed bacilli. Of all these vaccines, BCG has been the most extensively tried, and approximately 50 million persons have been vaccinated in many parts of the world. In spite of this large number of vaccinations, the effectiveness of BCG in increasing resistance is still a matter of controversy.

One of the great practical limitations of BCG vaccine is its very short life, as in general it should be used within ten days of its preparation and this period hardly allows for proper testing of sterility and antigenicity. This is one of the reasons why the preparation of this vaccine has been limited to a few laboratories and has not been attempted by commercial pharmaceutical companies.

The purpose of this paper is to present experience with a vaccine prepared by Parke, Davis and Company from strains of tubercle bacilli of human origin. The bacilli are killed by ultraviolet irradiation and, according to animal studies, the vaccine retains its antigenic properties for a long period of time without special storage precautions.[1]

One hundred children were inoculated with the irradiated vaccine (tables 1–3). These children, all of tuberculous parents, were born at the Hospital Alfredo J. Valenzuela in Guayaquil, Ecuador. In order to separate them from their parents, the children were all admitted to the infant's ward at Belen del Huerfano, where the study was conducted. These children were later returned to their homes after their parents had received proper care and were no longer infective and when the children themselves were in good nutritional condition and had been inoculated against tuberculosis. Previous to this study fresh BCG vaccine was used for the purpose of inoculation.

The children remained in the hospital for at least six weeks after vaccination in order to allow time for development of immunity. In spite of the fact that these children came from very poor environments and at least one of their parents had tuberculosis, the mortality rate while in the hospital was relatively low. During 1954 it was 5 per cent and during 1955 it was 2 per cent.

The irradiated tuberculosis vaccine is prepared[1] by growing a highly virulent human strain of *Myobacterium tuberculosis* for ten to twelve days on a liquid synthetic medium, grinding with glass beads to break up clumps, filtering through nylon to produce an even suspension and, after diluting to 1 mg. semi-moist weight of organisms per ml., irradiating with an ultraviolet lamp. After removing samples for *in vitro* and *in vivo* sterility and safety tests, merthiolate is added to make a 1 in 10,000 concentration. Stability tests indicate no apparent loss of potency after more than three years of storage. In experimental[1] work with more than 300 guinea pigs evaluated by the Feldman Index, which assigns arbitrary values to the microscopic evidence of the tuberculosis in the spleen, liver, and lungs, it was

[1] Sarber, R. W., *et al.*: Am. Rev. Tuberc., 1950, *62*, 418.

TABLE 1

VACCINATION WITH IRRADIATED TUBERCULOSIS VACCINE
MANTOUX REACTION REMAINED NEGATIVE

Dosage	Number Vaccinated	Time Involved (Follow-up)
0.25 ml. × 3	1	3 months
0.25 ml. × 1 0.50 ml. × 2 1.00 ml. × 1	1	6½ months
0.25 ml. × 1 1.50 ml. × 3 1.00 ml. × 1	1	13½ months
0.25 ml. × 1 0.50 ml. × 2 1.00 ml. × 2	1	12½ months
0.50 ml. × 2 1.00 ml. × 1	1	3 months, 1 week
0.50 ml. × 4	1	5 months
0.50 ml. × 1 1.00 ml. × 1	2	2½ months, 6 months
0.50 ml. × 2	1	1½ months
0.50 ml. × 1 1.00 ml. × 3	1	9½ months
0.50 ml. × 1 1.00 ml. × 2	5	3 months, 8 months, 11½ months, 9 months, 6 months
1.00 ml. × 1	13	4 months, 1 month, 20 days, 6 months, 1½ months (2)*, 4½ months, 3 months (4), 3½ months, 2½ months.
0.75 ml. × 1 1.00 ml. × 1	1	3½ months
1.00 ml. × 2	14	2 months (2), 4 months, 1½ months (5), 3 months (2), 6½ months, 5½ months, 5 months (2)
1.00 ml. × 3	18	9 months, 4 months (3), 12 months, 10½ months, 11½ months, 2½ months (3), 2 months, 3½ months (3), 8 months, 6 months (2), 6½ months

* Figures in parentheses indicate number vaccinated (when more than one) for each time involved in follow-up.

found that the animals inoculated with irradiated vaccine had an index of 32. A parallel group vaccinated with BCG had an index of 36, and the unvaccinated control group had an index of 75. The theoretical maximal disease is represented by an index of 100.

At the beginning of this study, a dose of 0.25 ml. was given to children at least two months old. As no reaction was observed, younger infants were vaccinated and the dosage was progressively increased. As many as six injections were given to some children with-

out producing any reaction. The last 50 patients of this series received three 1 ml. doses two weeks apart, starting in most cases during the first five days of life.

Before each inoculation, except when the first injection was given only a few days after birth, a tuberculin test was done with 0.1 ml. containing 0.001 mg. of PPD.

TABLE 2

VACCINATION WITH IRRADIATED TUBERCULOSIS VACCINE
MANTOUX REACTION BECAME POSITIVE

Dosage	Number Vaccinated	Time Involved (Follow-up)
0.25 ml. × 1 0.50 ml. × 2	1	3 months
0.25 ml. × 1 0.50 ml. × 1 1.00 ml. × 1	1	4 months
0.25 ml. × 4	1	10 months
0.25 ml. × 1 0.50 ml. × 3	1	8 months
0.25 ml. × 2 0.50 ml. × 2	1	6½ months
0.25 ml. × 2 0.50 ml. × 3 1.00 ml. × 1	1	5 months
0.25 ml. × 2 0.50 ml. × 2 1.00 ml. × 1	1	13 months
0.50 ml. × 2 1.00 ml. × 1	3	12 months, 3 months, 8 months
0.50 ml. × 2 1.00 ml. × 2	1	11½ months
0.50 ml. × 1 1.00 ml. × 1	7	2 months, 11 months, 3½ months, 2½ months, 4½ months (3)*
0.50 ml. × 1 1.00 ml. × 3	1	17 months
0.50 ml. × 1 1.00 ml. × 2	3	8 months, 5½ months (2)
0.50 ml. × 1	1	1½ months
1.00 ml. × 1	2	4 months (2)
1.00 ml. × 2	1	8½ months
1.00 ml. × 3	2	9½ months, 7 months

* Figures in parentheses indicate number vaccinated (when more than one) for each time involved in follow-up.

TABLE 3
VACCINATION WITH IRRADIATED TUBERCULOSIS VACCINE
MANTOUX TEST NOT DONE

Dosage	Number Vaccinated
0.5 ml. × 1	1
1.0 ml. × 1	8
1.0 ml. × 2	2

Evaluation of any antituberculous vaccine is very difficult. The only accurate way to appraise the degree of protection conferred is to observe the incidence of tuberculosis in the vaccinated group and compare it with that in a similar control group.

In some instances the tuberculin reaction has been used as an indirect indication of immunity, assuming that the positive reactors had an increased resistance. It is generally accepted that this is true for the positive tuberculin reaction that follows a healed primary infection. It is doubtful that this same yardstick can be used to measure immunity following vaccination. Sarber and Hemans[3] have shown in animal experiments that there is no correlation between tuberculin sensitivity and immunity after vaccination and that, consequently, the tuberculin reaction is not a suitable quantitative measure of immunity for antituberculous vaccine assay.

Furthermore the incidence of tuberculosis will be influenced by such factors as the susceptibility, the degree of exposure, and the social and economic position of the patient. Incidence of the disease in a particular country, or even in the world as a whole, varies and has lately diminished steadily.

These described difficulties indicate that, in order to draw conclusions as to the efficacy of an antituberculous vaccine, large numbers of patients should be treated and carefully compared with similar control groups. The present writer is not in a position to conduct such a study and realizes that no conclusions can be drawn from the limited number of patients inoculated. It is believed, however, that it is worth while to report these first experiences with this new antituberculous vaccine. If the animal work with this vaccine is confirmed in humans, it possesses two very distinct advantages, namely, stability which allows time for adequate safety and testing, transportation and storage; and production of a low degree of tuberculin sensitivity, which permits repeated reinoculations.

Of the 100 infants vaccinated, 71 have returned to their homes and 29 still remain under observation in the Hospital Belen del Huerfano. Consequently, a fair number of the total group has been exposed to tuberculous patients after having been vaccinated. These patients have been followed with periodic physical examinations and roentgenograms, some for as long as two years, and no evidence of active tuberculosis has been discovered.

The vaccine was well tolerated and no local reactions were observed. This was in contrast to the previous experience with BCG which, in many instances, led to enlargement of the satellite lymph nodes and even suppuration.

No systemic reactions were observed from the irradiated vaccine and the procedure did not interfere with the normal gain of the infants' weight. Several of the children with positive tuberculin reactions received 1 ml. of irradiated vaccine without untoward effects. This would be associated with some danger if done with BCG.

Only 28 of the patients became tuberculin positive after vaccination (table 2) and in

[3] SARBER, R. W., AND HEMANS, M. J.: Am. Rev. Tubere., 1952, *66*, 351.

several instances this positive reaction was elicited after they had returned to their homes. As a consequence, it is possible that certain of these instances represent primary infections, although no roentgenographic evidence of such infection was observed. In most of these 28 infants, the tuberculin reaction was only mildly positive, the diameter of the papule not exceeding 5 or 6 mm. A low degree of tuberculin sensitivity and the fact that the children with positive tuberculin tests did not show any untoward reactions represent definite advantages because they would allow repeated vaccinations, thereby increasing the immunity.

In summary: From the experience gained with a vaccine composed of irradiated human tubercle bacilli, it may be assumed that the vaccine is low in toxicity and produces a minimum of allergic phenomena. The vaccine produces no local scarring. It also has the desirable feature of being stable for a considerable time, thus allowing ample time for transportation and storage before it is used.

None of the vaccinated children developed adenitis (either local or general) due to the vaccine. There were only 6 cases of ulceration at the site of inoculation. These apparently were produced by staphylococci and disappeared with appropriate antimicrobial therapy. BCG vaccination produces adenitis of the lymph nodes which drain the region of vaccination, whereas the irradiated vaccine produced no such changes. Moreover, the irradiated vaccine had no adverse influence on the general condition of the infants. All of the newborn were vaccinated on the third day of life, except those with intercurrent illness or those who were congenitally weak. These children were not vaccinated until they had reached a normal state.

The chest roentgenograms of the vaccinated children revealed no abnormalities of lung or lymph nodes.

Curves of body weight were in accordance with the general satisfactory condition of the vaccinated children. Most of the increments corresponded to the curve of normal increase according to age. The few times that the increment in weight stopped was due to enteric disease but, once the infection was eliminated, the children reacted satisfactorily.

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RELIABILITY OF A MICROBIOLOGIC ASSAY TECHNIQUE FOR MEASURING LOW CONCENTRATIONS OF ISONIAZID[1]

Orally administered isoniazid is extensively and rather rapidly converted to several metabolic derivatives which possess virtually no antimicrobial activity against tubercle bacilli.[2] So extensive is this metabolic degradation that as little as 1 per cent of a test dose may be excreted in the urine as unaltered (free) isoniazid. Moreover, the antimicrobial activity of isoniazid is attributable mostly, if not entirely, to this unmetabolized fraction.

Concentrations of biologically active, i.e., free, isoniazid in the blood of humans are frequently less than 1 γ per ml. of serum. Unfortunately, practical chemical techniques are not yet available for measuring these small quantities of active drug. For this reason, the present writers have used a microbiologic assay technique to measure concentrations of biologically active isoniazid in human serum. The present paper demonstrates the reliability of that method.

The method is a slight modification of that devised by Middlebrook and associates[3] and is as follows:

(1). Blood is collected aseptically and allowed to clot, and the serum is transferred to a sterile, stoppered test tube. It was found that serum might be stored in the frozen state for as long as twenty-one weeks without demonstrable effect upon drug concentration (table 2).

(2). Two milliliters of serum are transferred to a tube containing 8 ml. of synthetic culture medium *without albumin*. Serial dilutions are made by successively transferring 5 ml. aliquots into a series of tubes containing 5 ml. of synthetic medium *with albumin*. The final dilutions are 1:5, 1:10, 1:20, 1:40, 1:80, 1:160, 1:320. The medium used is the basic medium of Cohn and co-workers[4] without the pyridoxine or biotin, and with 0.5 per cent albumin and 2 γ of para-aminobenzoic acid per ml. of medium. Twenty-five units per ml. of penicillin are added to minimize contamination.

(3). Each tube is then inoculated with 0.05 ml. of a seven- to ten-day-old Tween®-albumin culture of isoniazid-susceptible streptomycin-resistant *M. tuberculosis* strain H37Rv. To prepare inocula, the culture is diluted 1:1 with Tween-albumin medium.

(4). As a reference standard, used each time the test is set up, a series of tubes of the same culture medium containing 0, 0.005, 0.01, 0.02, 0.04, 0.08, and 0.1 γ of isoniazid per ml. are inoculated in the same fashion with the test strain of H37Rv.

(5). The tubes are incubated at 37° C. for seven days.

Inhibition of growth is determined macroscopically by the absence of visible bacillary flocculation. When the end point is not precise, a loopful from each tube in question is stained by the Ziehl-Neelsen method and inhibition of growth is read microscopically.

Results are computed in the following manner: The lowest concentration of drug causing inhibition of growth is determined from the series of control tubes (step 4). Inhibition of growth in the tubes containing the serum specimen must be due to a similar concentration of drug. Thus, in order to obtain the serum concentration of isoniazid, the reciprocal of the greatest serum dilution showing growth inhibition is multiplied by the lowest drug concentration which inhibited growth in the control tubes. For example, if growth occurred in the first four control tubes but not in the fifth, i.e., no growth in 0.04 γ per ml., and if growth inhibition was observed in the first five tubes of the serum series, i.e., through 1:80 dilution, then the results would be 0.04 × 80, or 3.2 γ per ml. of serum.

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[2] HUGHES, H. B.: J. Pharmacol. & Exper. Therap., 1953, *109*, 444.

[3] MANDEL, W., *et al.*: Proc. Soc. Exper. Biol. & Med., 1956, *91*, 409.

[4] COHN, M. L., *et al.*: Am. Rev. Tuberc., 1954, *70*, 465.

TABLE 1
RELIABILITY OF BIOASSAY METHOD WITH AQUEOUS SOLUTIONS OF ISONIAZID

Sample Number	Isoniazid Concentration (γ/ml.) as Determined by:			Predicted Bioassay Level
	Weight-Dilution	Chemical Test (Kelley-Poet)	Bioassay	
				γ/ml.
1	0.25	0.30	0.2	0.2
2	0.31	0.37	0.4	0.2
3	0.42	0.48	0.4	0.4
4	0.47	0.53	0.4	0.4
5	0.50	0.68	0.4	0.4
6	0.63	0.72	0.8	0.4
7	0.83	0.99	0.8	0.8
8	0.94	1.04	0.8	0.8
9	1.00	1.04	0.8	0.8
10	1.25	1.30	0.8	0.8
11	1.67	1.76	1.6	1.6
12	1.88	1.90	1.6	1.6
13	2.00	2.11	1.6	1.6
14	2.50	2.50	3.2	1.6
15	3.33	3.34	3.2	3.2
16	3.75	3.63	3.2	3.2
17	4.00	4.00	3.2	3.2
18	5.00	4.71	3.2	3.2
19	6.67	6.45	6.4	6.4
20	7.50	7.35	6.4	6.4

TABLE 2
REPRODUCIBILITY OF RESULTS OBTAINED ON ALIQUOTS OF SINGLE SERUM SPECIMENS

Specimen	Bioassay of Serum Isoniazid (γ/ml.)			
	Aliquot A Tested 7-5-55	Aliquot B Tested 7-12-56	Aliquot C Tested 10-3-56	Aliquot D Tested 12-3-56
1	1.6	1.6		
2	3.2	3.2	3.2	3.2
3	3.2	3.2	3.2	3.2
4	3.2	3.2	3.2	3.2

In assaying the aqueous solutions of isoniazid, the test is performed in the same manner, except that medium containing albumin is used in all tubes.

It was recognized at the beginning of these studies that the presence of human serum in the unknown series of tubes might have some effect upon the end point of growth inhibition of the test strain of organisms. For this reason human serum containing no isoniazid was added to one of a duplicate series of standards (step 4) in the same way as was done with the unknown (step 2). The presence of human serum had no detectable effect. Similar observations have been made by Middlebrook[5] and by Morse.[6]

[5] MIDDLEBROOK, G.: Personal communication.
[6] MORSE, W. C.: Personal communication.

One of the obvious limitations of this method is that the scale of measurement is not in continuous or equal increments; results are expressed as 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, or 12.8 γ per ml., and the measurement of values intermediate between these concentrations is not practical. For example, a serum specimen containing 3.0 γ per ml. would be read as containing only 1.6 γ per ml. by this method, whereas serum specimens containing from 3.3 to 6.0 γ per ml. would all yield a bioassay value of 3.2, et cetera.

It therefore seemed advisable to ascertain what values actually would be obtained by the bioassay technique with a series of aqueous solutions containing various known concentrations of isoniazid which ranged from 0.2 to 7.5 γ per ml.

An aqueous solution containing a carefully weighed amount of isoniazid (10 γ per ml.) was prepared, and sterilized by passing through a Seitz filter. Chemical analysis by the Kelley-Poet technique[1] before and after filtering revealed no detectable effect on the concentration of isoniazid. Several concentrations, i.e., 7.5, 6.67, 5.0, and 4.0 γ per ml., were prepared as stock solutions. From each of these 4 stock solutions, 5 serial dilutions were made, using aseptic technique, and on the resulting 20 dilutions both the bioassay and the chemical tests were performed. These solutions were prepared and coded by the chemist, given to the bacteriologist, and the bioassay results were read by a third person who had no knowledge of what the concentrations were. The results are presented in table 1.

In 17 of the 20 dilutions the bioassay method yielded the exact result which was anticipated on the basis of the chemically measured content. In only 3 of the 20 samples did the bioassay method fail to yield the predicted values. In 2 of these (*Nos. 2* and *6*), the error was less than 0.2 γ per ml., well within the range of deviation exhibited by the chemical method.

Another question to be answered was whether or not the test would yield reproducible results when performed at different times on aliquots of single serum specimens containing identical concentrations of drug.

Serum specimens were collected from 4 normal subjects two hours after the oral administration of a test dose of isoniazid. Each of the specimens was divided into four aliquots. Bioassays were run immediately on one of the aliquots, and the other three aliquots were frozen. These aliquots were defrosted and tested by the bioassay method at various intervals up to twenty-one weeks, as indicated in table 2. In every instance the separate determinations on aliquots of each serum specimen yielded identical results.

It is concluded that this microbiologic assay method for measuring isoniazid is a reliable and reproducible procedure when carried out with proper precautions and attention to detail.

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[1] KELLEY, J. M., AND POET, R. B.: Am. Rev. Tuberc., 1952, *65*, 484.

USE OF A SERUM MICROBIOLOGIC ASSAY TECHNIQUE FOR ESTIMATING PATTERNS OF ISONIAZID METABOLISM[1]

Hughes[2] has demonstrated by reliable chemical techniques that the pattern of metabolic degradation of isoniazid is surprisingly constant in a given individual, although it varies widely from person to person. These observations were made by measuring the amounts of free, i.e., unaltered, isoniazid and its metabolites excreted in the urine after the oral administration of a single test dose of drug. Unfortunately, the chemical method used to measure the biologically active fraction, i.e., free, unaltered isoniazid, in the urine is not sensitive enough to measure the small amounts of free drug encountered in human serum. However, recent investigations[3-5] have demonstrated the reliability of microbiologic assay methods for measuring low concentrations of biologically active isoniazid.

The purpose of this investigation was to determine whether or not serum concentrations of biologically active drug, as measured by a microbiologic assay method, would reflect a constancy of metabolic pattern similar to that demonstrated by chemical techniques in the studies of urinary excretion.

The biologic assay technique is that described in a previous communication.[5] One of the limitations of bioassay techniques, when the samples are diluted serially, is that the units of measurement are not expressed in equal increments. Thus, the results are expressed as 0.2, 0.4, 0.8, 1.6, 3.2, et cetera, γ per ml. However, the use of such methods seems adequately justified in the absence of reliable chemical techniques.

Thirty subjects were chosen for study: 14 were laboratory and secretarial personnel working in the hospital and 16 were tuberculous patients in this hospital. In most subjects a single test dose of isoniazid was given on two or three consecutive days and, in many, another test dose was given on at least one other occasion. Sometimes the interval between repeat test doses was as long as six months and in some of the tuberculous subjects, all of whom were given chemotherapy (including isoniazid), there had been marked improvement in the general state of health during the interval between repeat tests.

The testing protocol was as follows:

Breakfast was omitted. Each test dose of isoniazid (4 mg. per kg. of body weight) was given in a single oral dose on an empty stomach at 7:00 a.m. A fat-free breakfast was given at 8:30 a.m. Blood specimens were drawn at two and six hours after drug administration. Some word of caution seems appropriate regarding the care with which this protocol must be followed. For instance, it was found that a normal fatty breakfast frequently was followed by erratic bioassay results. Similarly, it was found that taking food too soon after administration of the test dose may result in low serum concentrations of isoniazid.

The results are presented in table 1. It is apparent immediately that the general configuration of the curve of drug concentration in any one subject is reproducible from time to time, whether repeated on consecutive days or at intervals of several weeks or months. However, it is equally apparent that a one-tube variation was often observed in the serum

[1] This study was supported, in part, by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association, and by a contract (No. SAph 67795) with the U. S. Public Health Service.

[2] HUGHES, H. B.: J. Pharmacol. & Exper. Therap., 1953, *109*, 444.

[3] MORSE, W. C.: Personal communication.

[4] MANDEL, W., *et al.*: Proc. Soc. Exper. Biol. & Med., 1956, *91*, 409.

[5] BELL, J. C., AND RIEMENSNIDER, D. K.: Am. Rev. Tuberc., 1957, *75*, 992.

TABLE 1

Reproducibility of Serum Bioassay Concentrations of Isoniazid on Repeated Testing

Subject	Date	Serum Isoniazid (γ/ml.)		Subject	Date	Serum Isoniazid (γ/ml.)		Subject	Date	Serum Isoniazid (γ/ml.)	
		2 hours	6 hours			2 hours	6 hours			2 hours	6 hours
1	9-10-56	0.8	<0.2	10	9-10-56	3.2	1.6	21	8-1-56	3.2	1.6
	9-11-56	0.8	<0.2		9-11-56	6.4	1.6		10-4-56	6.4	1.6
	9-12-56	0.8	<0.2		9-18-56	3.2	1.6		10-5-56	3.2	1.6
	10-3-56	0.8	<0.2								
	10-19-56	0.8	<0.2	11	9-10-56	3.2	1.6	22	9-24-56	1.6	0.8
					9-11-56	1.6	0.8		10-4-56	3.2	0.8
2	9-10-56	0.8	<0.2		9-12-56	3.2	1.6		10-5-56	3.2	1.6
	9-11-56	0.8	<0.2								
	9-12-56	0.8	<0.2	12	10-3-56	0.8	0.2	23	9-11-56	3.2	0.8
	10-3-56	0.8	<0.2		10-4-56	1.6	<0.2		10-4-56	1.6	0.8
	10-19-56	0.4	<0.2		10-6-56	1.6	0.2		10-5-56	3.2	0.8
3	7-5-56	3.2	—	13	10-3-56	6.4	0.8	24	7-23-56	0.8	0.2
	9-10-56	3.2	0.8		10-4-56	3.2	1.6		10-2-56	1.6	0.2
	9-11-56	3.2	0.8		10-6-56	6.4	1.6				
	9-12-56	3.2	0.8					25	9-6-56	3.2	0.4
				14	3-20-56	3.2	0.8		10-8-56	1.6	<0.4
4	9-10-56	3.2	0.8		7-5-56	1.6	—				
	9-11-56	3.2	0.8		9-10-56	1.6	0.8	26	7-23-56	1.6	0.4
	9-12-56	3.2	0.8		9-11-56	3.2	0.8		9-?-56	—	0.4
	10-3-56	3.2	1.6		9-12-56	1.6	0.2				
					10-3-56	3.2	0.8	27	9-24-56	3.2	0.8
5	9-10-56	0.8	<0.2						10-4-56	3.2	1.6
	9-11-56	0.8	<0.2	15	10-2-56	3.2	1.6				
	9-12-56	0.4	<0.2		10-3-56	3.2	1.6	28	7-20-56	1.6	0.8
	10-3-56	0.8	<0.2		10-4-56	3.2	0.8		8-13-56	3.2	0.8
6	7-5-56	3.2	—	16	9-24-56	1.6	0.2	29	8-9-56	3.2	0.4
	9-10-56	3.2	1.6		10-4-56	1.6	0.4		9-11-56	3.2	0.8
	9-11-56	3.2	0.8		10-5-56	1.6	0.2				
	9-12-56	3.2	0.8					30	4-3-56	3.2	0.4
	10-3-56	3.2	1.6	17	10-1-56	0.8	0.2		8-13-56	3.2	0.4
	10-19-56	3.2	0.8		10-2-56	1.6	0.4				
					10-4-56	0.8	0.2				
7	7-5-56	3.2	—								
	9-10-56	3.2	0.8	18	10-1-56	0.8	<0.2				
	9-11-56	3.2	0.8		10-2-56	0.8	0.4				
	9-12-56	3.2	1.6		10-3-56	1.6	0.2				
8	9-10-56	1.6	0.8	19	10-1-56	1.6	0.4				
	9-11-56	3.2	1.6		10-2-56	3.2	0.4				
	9-12-56	3.2	1.6		10-3-56	3.2	0.4				
9	9-10-56	0.4	<0.2	20	10-1-56	6.4	1.6				
	9-11-56	0.8	0.2		10-2-56	3.2	1.6				
	9-12-56	0.8	<0.2		8-21-56	contaminated	1.6				

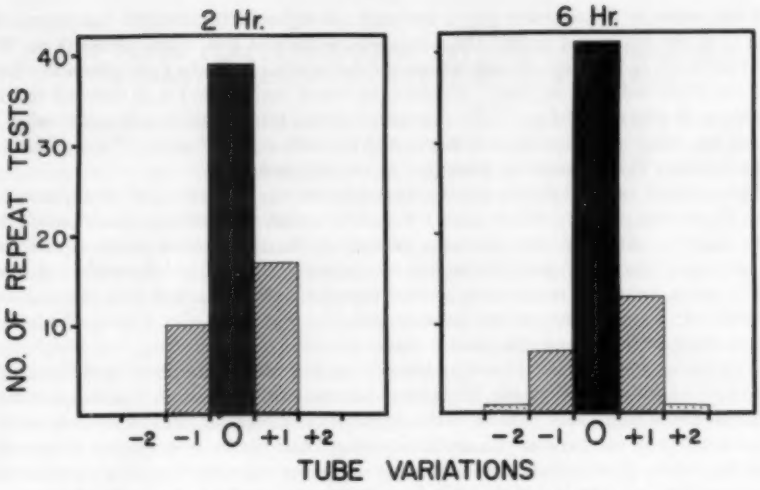

FIG. 1. Variation of serum bioassay determinations in 30 persons.

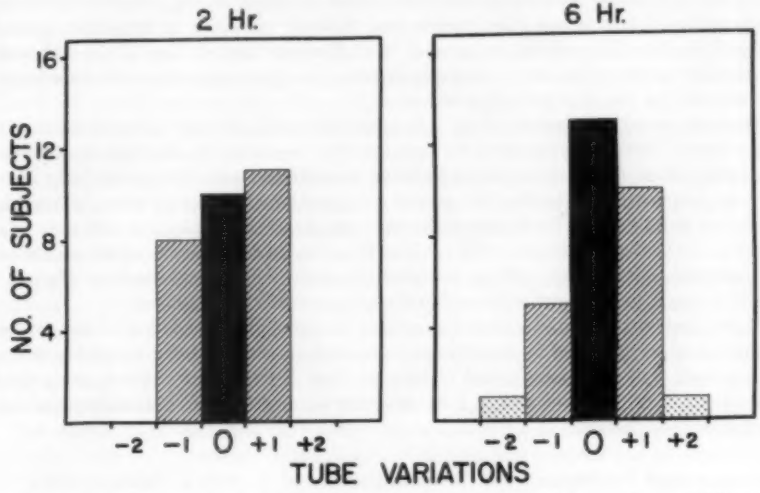

FIG. 2. Maximal variation of metabolic pattern as measured by repeat serum bioassay determinations in 30 persons.

concentration of biologically active drug, e.g., variation from 0.8 to 1.6, or from 1.6 to 3.2, et cetera. Only rarely did the variation in serum concentration exceed a one-tube difference.

In an effort to evaluate more critically this variation, the results in table 1 were subjected to the following analysis. The first bioassay determination performed on each sub-

ject was taken as the reference point, and each subsequent determination was compared with it. If the repeat test yielded the same value as the first test, it was recorded as "0" (no variation). If, however, the repeat test yielded a value one tube higher than the first run, i.e., if the end point of growth inhibition increased from 0.8 to 1.6, or from 1.6 to 3.2, et cetera, it was recorded as "+1" (variation of one tube greater); and conversely, a repeat run value one tube lower than the first test was recorded as "−1" (variation of one tube less). These results are presented graphically in figure 1.

The protocol, outlined above, was repeated sixty-six times for two-hour serum samples from 30 persons, and sixty-three times for six-hour serum samples from these same subjects (figure 1). At two hours, 39 repeat tests yielded bioassay values identical with the original observations; 17 gave results one tube greater, and 10 yielded results one tube lower. At six hours, 41 repeat tests yielded bioassay values identical with the original observation; 13 gave results one tube greater, one was two tubes greater, 7 yielded results one tube lower, and one was two tubes lower.

A similar procedure was followed in determining the extent and direction of the maximal variation for each individual. The initial observation on each subject again was taken as the reference point; then from all of the repeat tests on each subject the maximal variation was taken for comparison with the initial value. These results are presented in figure 2.

At two hours, 10 subjects demonstrated no variation on repeat testing, 11 showed serum concentrations one tube higher than baseline observations, and 8 had a maximal variation of one tube lower. (Note: Subject *No. 26* did not have a repeat two-hour observation.) At six hours, 13 subjects demonstrated no variation on repeat testing; 10 subjects showed a maximal variation of one tube greater; one showed a variation of two tubes greater (Subject *No. 18*—the maximal variation of two tubes was from <0.2 to 0.4, a difference in the range of <0.4 γ per ml.); 5 subjects showed a maximal variation of one tube lower; and one subject (*No. 14*), two tubes lower.

Thus the metabolic pattern of an individual, following the oral administration of a single dose of isoniazid, appears to be constant when measured by the bioassay method. On repeat testing, a one-tube variation in serum isoniazid concentration occurs fairly often, but this does not appear to alter the general configuration of the serum curve. The variation could well be due to limitations inherent in the bioassay technique as well as to relatively minor biologic variation in the subjects from day to day. In spite of this variation, the metabolic pattern, especially as reflected by six-hour drug concentrations (figures 1 and 2), appears to be reproducible within the range of chance deviations.

These observations indicate that the pattern of metabolic degradation of isoniazid in an individual, as measured by microbioassay, is constant not only from day to day during brief periods, but also over several months at least. Tuberculous patients, in spite of lessening of fever, anorexia, weight loss, and other signs of toxicity, also maintain a constant metabolic pattern.

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SERUM PAPER ELECTROPHORETIC PATTERNS OF TUBERCULOUS PATIENTS PRESENTING THERAPEUTIC PROBLEMS

The literature reflects an interest in the electrophoretic pattern of the serum proteins in tuberculosis, dating back to 1942 when Seibert and Nelson[1] reported a rise in the gamma globulin level in minimal tuberculosis which continued to increase with the severity of the disease. In far advanced tuberculosis they noted an increase in all of the globulins. In the following year[2] these same writers again reported on the serum protein changes in human tuberculosis and compared the pattern observed with that in experimentally infected tuberculous rabbits. They found a consistent decrease in albumin and an increase in beta globulin in the rabbits, while the human patients showed a decrease in albumin and an increase in alpha and gamma globulin concentration. In 1947, Seibert and associates[3] correlated the serum protein patterns with the stage of the tuberculous disease process. They reported a rise in gamma globulin and a fall in albumin concentration in minimal, active tuberculosis. In moderately advanced disease they observed, in addition to the above, an increase in the alpha-2 globulin fraction which they related to the increased polysaccharide content of the serum. In far advanced tuberculosis, they observed a further increase in all of the above changes together with an increase in all of the globulins.

In 1953, Volk and co-workers[4] confirmed these earlier reports and observed that alpha-2 and beta globulins remain elevated even in arrested cases. Baldwin and Iland,[5] in 1953, reported an increase in the alpha-1, alpha-2, and gamma globulin fractions which paralleled the progress of the disease. This increase was accompanied by a corresponding decrease in the albumin fraction. Although they demonstrated the presence of complement-fixing antibodies in the gamma globulin fraction, they could detect no change in the concentration of this fraction when tuberculous serum was absorbed with *M. tuberculosis*, and they concluded that most of the increased gamma globulin detected in the serum of tuberculous patients is not antibody.

In 1954, Kanagami[6] reported his observations from a study of the serum proteins in 122 patients with pulmonary tuberculosis untreated chemotherapeutically. In general, he confirmed the earlier observations except for beta globulin which he noted showed no correlation with the disease process.

In 1955, the technique of paper electrophoresis was applied to this problem. Matsui and Tamura[7] confirmed the correlation between the decreased albumin, increased globulin fraction, and the severity of the disease. They also noted a smaller beta globulin fraction in far advanced tuberculosis than in moderately advanced cases. Malargia and Gelli[8] noted a decrease in albumin and an increase in alpha and gamma globulins in tuberculous patients subjected to thoracoplasty or extrapleural pneumothorax.

The present paper describes the results of an investigation of the paper electrophoretic patterns of the serum proteins in the case of 31 tuberculous patients and 19 normal subjects. Eleven of the patients are considered, clinically, to be problems in therapy and are hereafter referred to as the "therapeutic problem" group.

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[1] Seibert, F. B., and Nelson, J. W.: J. Biol. Chem., 1942, *143*, 29.
[2] Seibert, F. B., and Nelson, J. W.: Am. Rev. Tuberc., 1943, *47*, 66.
[3] Seibert, F. B., *et al.*: J. Clin. Investigation, 1947, *26*, 90.
[4] Volk, B. W., *et al.*: Am. Rev. Tuberc., 1953, *67*, 299.
[5] Baldwin, R. W., and Iland, C. N.: Am. Rev. Tuberc., 1953, *68*, 372.
[6] Kanagami, H.: Sc. Rep. Research Inst., Tohoku Univ., 1954, *5*, 415.
[7] Matsui, K., and Tamura, S.: Kekkaku, 1955, *30*, 563.
[8] Malargia, A., and Gelli, G.: Arch. "E. Maragliano" pat. e clin., 1955, *11*, 309.

TABLE 1

STATISTICAL SUMMARY OF THE RESULTS SHOWING THE SIGNIFICANT DIFFERENCES BETWEEN THE GROUPS FOR EACH OF THE FIVE PROTEIN FRACTIONS

	Group	Mean	Observed Range	Selected Range	Per Cent of N*
Albumin	*N*	*60.1*	*54.4–70.2*	*53.0–67.0*	*97.2*
	A	60.4	51.5–67.0	50.4–70.4	90.0
	B	50.6	37.2–62.0	35.0–66.2	30.0
	C	46.0	34.8–54.6	35.1–56.9	9.1
Alpha-1 globulin	*N*	*3.4*	*1.6—6.0*	*1.4—5.4*	*97.2*
	A	3.4	2.5—4.8	2.9—3.9	100.0
	B	4.8	2.8—7.9	1.9—7.7	70.0
	C	4.5	2.9—6.0	2.4—6.6	72.7
Alpha-2 globulin	*N*	*8.2*	*4.5–12.6*	*4.6–11.8*	*91.7*
	A	9.2	5.7–12.2	4.6–13.8	90.0
	B	13.2	8.9–17.6	7.7–18.7	40.0
	C	11.6	8.6–14.4	8.3–14.9	63.6
Beta globulin	*N*	*13.9*	*9.6–17.1*	*10.1–17.7*	*97.2*
	A	11.2	9.9–13.2	9.0–13.4	90.0
	B	13.7	7.5–22.4	5.7–21.7	80.0
	C	15.0	10.2–21.2	8.9–21.1	81.8
Gamma globulin	*N*	*14.5*	*9.2–18.4*	*10.1–18.9*	*97.2*
	A	15.8	10.7–23.4	7.1–24.5	70.0
	B	17.7	10.9–22.5	10.7–24.7	70.0
	C	22.9	15.3–28.8	14.4–31.4	18.2

* Percentage of patients in each group having levels in the selected normal range.

Three groups of tuberculous patients were studied. The "therapeutic problem" group consisted of 11 *Class I* and *Class III*[9] patients who had been treated for one or more years with varied chemotherapeutic regimens. Tubercle bacilli had persisted in the sputum and there had been no demonstrable improvement in the roentgenograms. The "pre-therapy" group was composed of 10 *Class II* patients who had received less than two months of therapy. The extent of tuberculous disease in this group ranged from minimal through far advanced. No consideration is given to these differences in the study because the purpose which the group serves here is simply to provide a reference for comparison with the other two groups. The "post-therapy" group consisted of 10 *Class IV* tuberculous patients who were clinically considered to have responded successfully to chemotherapy. In all cases, the sputum was negative for tubercle bacilli and the roentgenograms were free of active tuberculous lesions. The normal group consisted of 19 apparently normal laboratory volunteers.

The blood specimens in this study were all drawn at least three hours post prandium. No preservatives were employed and the serum was separated from the clot one to three hours after collection. A commercial model[10] of the Durrum "hanging strip" type electrophoresis cell was employed with a constant current of 5 milliamperes for sixteen hours

[9] Diagnostic Standards and Classification of Tuberculosis, 1950 edition. National Tuberculosis Association, New York.

[10] Spinco Model R Paper Electrophoresis Apparatus, Spinco Division, Beckman Instruments, Inc., Belmont, California.

at room temperature. A barbital buffer with a pH of 8.6 and an ionic strength of 0.075 was employed. Ten microliters (0.01 ml.) of serum were applied to the strip in a band at the apex. The electrophoretic protein patterns so obtained were dried for twenty minutes in an oven at 120°C., stained with bromphenol blue and zinc sulfate, and fixed with sodium acetate.

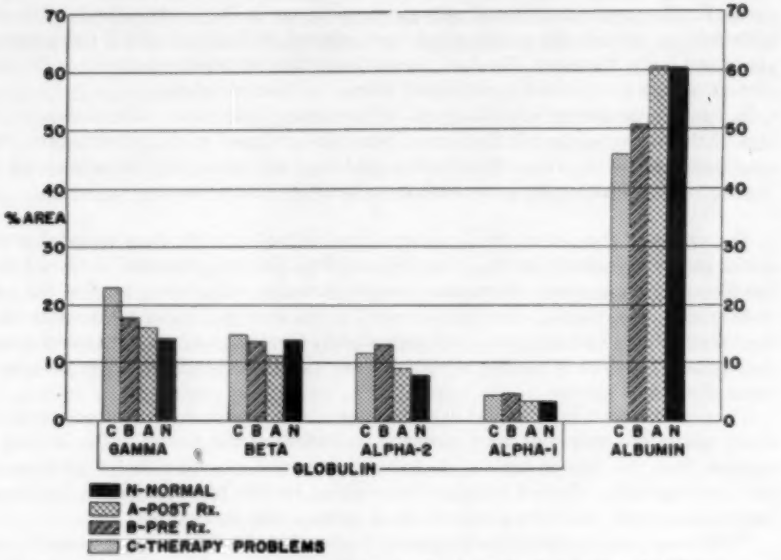

FIG. 1. Comparing the mean group values for each of the five protein fractions.

TABLE 2

COMPARISON OF THE FINDINGS IN THIS STUDY WITH THOSE REPORTED IN THE LITERATURE REVIEWED

Literature Reference	Year	Albumin	Alpha 1	Alpha 2	Beta	Gamma
Seibert and Nelson[1]	1942	N	N	N	N	+
Seibert and Nelson[2]	1943	−	+	+	N	+
Seibert *et al.*[3]	1947	−	+	+	+	+
Volk *et al.*[4]	1953	−	+	Sustained + PT	Sustained + PT	+
Baldwin and Iland[5]	1953	−	+	+	N	+
Kanagami[6]	1954	−	+	+	0	+
Matsui and Tamura[7]	1955	−	+	+	−ϕ	+
Maher *et al.*	1956					
A. Post-therapy group		N	N	N	N	+
B. Early therapy group		−	+	+	+	+
C. Therapeutic problem group		−−	+	+	+	++

N: normal; +: elevated; ϕ: far advanced tuberculosis; ++: grossly elevated; −: depressed; 0: no correlation with disease process; −−: grossly depressed; PT: post therapy.

After allowing twenty-four hours for the dye to equilibrate with atmospheric moisture, the strips were scanned in the automatically integrating densitometer and the per cent distribution of the proteins was calculated.

The observations and conclusions are summarized in table 1. The range of values observed for the protein fractions of each group are shown in the fourth column, while the fifth column presents the ranges which were selected on the basis of a 2 sigma spread above and below the mean. The last column shows the conclusions expressed as the percentage of each group which is considered normal by these standards.

In figure 1 the groups are contrasted with respect to the mean value calculated for each of the five serum protein fractions. These data are based on the percentage of total area under each of the five protein fraction peaks and are arranged in the order in which the protein fractions appear in the pattern to facilitate comparison.

* * *

The results of the present study are compared in table 2 with those reported in the earlier literature reviewed above. It may be seen from the data presented in figure 1 that the therapy problem group shows more marked anomalies with respect to all of the fractions except alpha-1 and -2. The "pre-therapy" group showed a higher mean value while the "therapeutic problem" group, although slightly elevated, more closely approximated the normal. The data in the last column of table 1 confirm this observation, the significance of which is obscure.

The protein anomalies observed in this disease can be considered as another expression of the generalized metabolic degeneration characteristic of the disease. If this is done, it appears, from the data in table 1, that the albumin and gamma globulin fractions are the most markedly affected and that the albumin returns to normal when the disease process is arrested, while the gamma globulin remains slightly elevated.

With respect to the "therapeutic problem" group, the data of this study seem to indicate that the sera of these patients show a continued degeneration of the albumin with a concurrent elevation of the gamma globulin fraction. The differences in the beta fraction between the "pre-therapy" group and the "therapeutic problem" group are not considered statistically significant. The differences in the alpha globulin fractions have been discussed above.

In summary: The concentration of the serum protein fractions of 31 tuberculous patients was studied by means of paper electrophoresis and compared with a similar study of the sera from 19 apparently normal subjects. The tuberculous patients were divided into a "pre-therapy" and "post-therapy" group of 10 patients each and a "therapeutic problem" group of 11 patients. The resulting data were analyzed and compared with those reported in the literature. It is concluded that the anomalies noted in the case of the "therapeutic problem" group were simply an indication of a continuing degenerative process related to the disease. The alpha globulin fractions were exceptional in this respect.

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[11] The writers acknowledge with deepest gratitude the assistance of Mr. Edward D. Jenkins of this laboratory who is responsible for the statistical analysis of the data.

SOME BIOCHEMICAL ASPECTS OF BENZOYL PARA-AMINOSALICYLIC ACID METABOLISM

The effectiveness of para-aminosalicylic acid (PAS) as an adjuvant in isoniazid and streptomycin therapy has been firmly established. However, it is unpalatable to some patients, and certain unpleasant side effects are sometimes experienced. In the search for a more compatible form of PAS, the benzoyl derivative was synthesized and the insoluble calcium salt was prepared.

In conjunction with a clinical evaluation of calcium benzoyl para-aminosalicylic acid,[1] a portion of its metabolic course has been studied. Blood concentrations and excretion rates of benzoyl para-aminosalicylic acid (benzoyl PAS) and some of its metabolic products were determined and a study was made of the factors influencing the formation of meta-aminophenol.

The method available, at the clinical level, for the determination of benzoyl PAS products are relatively undeveloped. The available methods are based on the use of nonspecific reagents. Because benzoyl PAS and its metabolites are so similar in structure, more than a single compound is usually measured by any one method. Acid hydrolysis so commonly used in sulfonamide metabolism studies to deacetylate is in itself nonselective. As a result, the acetyl forms of meta-aminophenol, PAS, and benzoyl PAS are hydrolyzed. In addition, the latter two compounds are decarboxylated in varying degrees to meta-aminophenol.

The chemical analyses in this study were restricted to benzoyl PAS, PAS[2], and meta-aminophenol.[3] Benzoyl PAS and PAS do not react with the reagents used for the determination of meta-aminophenol. Prior to its estimation, benzoyl PAS was extracted from plasma (acid) with chloroform. PAS is not chloroform soluble. The benzoyl PAS fraction was determined by a new method developed in this laboratory, based on the alkaline hydrolysis of benzoyl PAS. The resulting PAS was estimated in the conventional way by diazotizing and coupling with N-(1-napthyl) ethylenediamine.

Three normal subjects (laboratory personnel) were given a single 4 gm. oral dose of benzoyl PAS. Plasma determinations on blood taken at intervals over a seven-hour period are listed in table 1. The expected variations between individuals occurred, due to differences in absorption and metabolism, the discrepancy between any two-hour samples being about twofold. The average peak concentration of benzoyl PAS was reached between two and four hours. The presence of PAS was detectable in the first sample taken one hour after ingestion of the drug. Although PAS was always present in plasma after benzoyl PAS, no meta-aminophenol could be found in several of the samples.

The control urine results for each component are expressed in milligrams per volume of urine excreted at the designated time (table 2). Only two subjects from the control group were included. A large proportion of benzoyl PAS is excreted unaltered and appears at least within an hour after administration. PAS is also present in significant quantities, indicative of benzoyl PAS hydrolysis. Between 1.3 and 1.7 gm. of the 4 gm. of calcium salt administered is excreted in seven hours as benzoyl PAS and PAS.

The data obtained by using single oral doses of 4 gm. of calcium benzoyl PAS are not sufficient to support the possibility of PAS decarboxylation to form meta-amenophenol.[3]

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[1] The calcium benzoyl PAS was supplied as $(BPAS)_2Ca$ by the Wander Company, Chicago, Illinois.

[2] Way, E. L., *et al.*: J. Pharmacol. & Exper. Therap., 1948, *93*, 368.

[3] Pesez, M.: Bull. Soc. Chim. biol., 1949, *31*, 1369.

Pesez's method for meta-aminophenol is capable of detecting the presence of a substance like meta-aminophenol in the urine of untreated persons. A group of 15 male and female children, four to thirteen years old, had an average meta-aminophenol concentration of 0.9 mg. per 100 ml. (range 0.6–1.4 mg. per 100 ml.). Twenty-two male and female adults had an average urine concentration of 1.5 mg. per 100 ml. (range 0.6–2.0 mg. per 100 ml.).

Each urine sample from a control subject receiving the calcium salt can be expected to contain some quantity of the normally occurring meta-aminophenol. In order to establish the significance of the meta-aminophenol values listed in tables 2, 3, and 4, the amount of normal urine meta-aminophenol was calculated for each urine volume, i.e., urine volume, ml. $\times$ 0.015. These values appear in parentheses. The difference between the determined and theoretic values of the controls (table 2) indicate that PAS is not decarboxylated to meta-aminophenol under the conditions of this particular experiment. Two of the control subjects were treated with 2.6 gm. of sodium PAS equivalent to 4 gm. of calcium benzoyl PAS at 8:00 a.m. and 12:00 noon. The plasma PAS and meta-aminophenol concentrations were similar to those in table 1 except for the two-hour PAS concentrations

TABLE 1

PLASMA CONCENTRATIONS OF BENZOYL PAS, PAS, AND META-AMINOPHENOL AFTER A SINGLE 4 GM. DOSE OF CALCIUM BENZOYL PAS

Time After Administration of Drug	Average of 3 Normal Controls, mg/100 ml.		
	Benzoyl PAS	PAS	Meta-Aminophenol
Fasting	0.0	0.0	0.0
1 hour	3.4	0.2	0.0
2 hours	4.8	0.4	0.1
3 hours	2.9	0.3	0.0
4 hours	3.4	0.2	0.1
7 hours	1.2	0.1	0.1

TABLE 2

EXCRETION OF BENZOYL PAS, PAS, AND META-AMINOPHENOL IN URINE AFTER A SINGLE 4 GM. DOSE OF CALCIUM BENZOYL PAS·5H_2O

Time Lapse After Drug Administration	Benzoyl PAS	PAS	Meta-Aminophenol	Volume of Urine Excreted
	mg.	*mg.*	*mg.*	*ml.*
Control 1, fasting	0		1.8(0.8)*	55
1 hour	97	38	2.8(1.1)	70
2 hours	171	56	2.9(1.0)	67
3 hours	158	43	3.6(2.5)	165
4 hours	131	35	5.1(6.3)	420
7 hours	143	20	3.5(2.3)	153
Control 2, fasting	0	0	1.3(2.0)	130
1 hour	140	28	0.0(12.0)	800
2 hours	96	21	1.2(1.4)	85
4 hours	470	96	4.2(7.3)	490
7 hours	323	45	4.2(2.9)	190

* The values in parentheses represent the theoretic amount of meta-aminophenol in the urine excreted, based on a normal average concentration of 1.5 mg. of meta-aminophenol per 100 ml. of urine.

TABLE 3

EXCRETION OF PAS AND META-AMINOPHENOL IN URINE AFTER TWO ORAL DOSES OF SODIUM PAS (2.6 GM. EACH)

Time Lapse After Drug Administration	Control 1		Control 2		Time of Drug Administration
	PAS	Meta-Aminophenol	PAS	Meta-Aminophenol	
	mg.	*mg.*	*mg.*	*mg.*	
Fasting	0	0(0.2)*	0	0.6(0.4)*	8:30
2 hours	422	—	336	0.6(1.0)	
4 hours	54	5.8(2.0)	100	15.6(1.0)	12:30
6 hours	481	29.5(1.8)	483	31.7(1.8)	
8 hours	71	15.8(3.3)	—	—	
13 hours	24	4.8(7.9)	—	—	
24 hours	9	5.3(9.0)	10	5.3(3.5)	

* The values in parentheses represent the theoretic amount of meta-aminophenol in the urine excreted, based on a normal average concentration of 1.5 mg. meta-aminophenol per 100 ml. of urine.

TABLE 4

AVERAGE PAS AND META-AMINOPHENOL CONCENTRATIONS IN PLASMA AND URINE IN TWO PATIENTS RECEIVING 4 GM. OF SODIUM PAS PER MEAL

Time Lapse After Drug Administration	PAS		MAP		Drug Administered
	Plasma	Urine Excreted	Plasma	Urine Excreted	
	mg./100 ml.	*mg.*	*mg./100 ml.*	*mg.*	
Fasting	0	3.8	0.0	1(0.6)*	8:00
2 hours	4.1	811	0.2	46(1.7)	
4 hours	1.7	400	0.4	30(1.2)	12:00
6 hours	5.2	908	0.2	48(1.5)	

* The values in parentheses represent the theoretic amount of meta-aminophenol in the urine excreted, based on a normal average concentration of 1.5 mg. of meta-aminophenol per 100 ml. of urine.

which ranged between 1 and 2 mg. per 100 ml. Large amounts of PAS were excreted unchanged. The urine collected between the fourth and eighth hours contained meta-aminophenol in amounts three to fifteen times greater than the theoretically calculated values (table 3).

The several patients studied were from the Pulmonary Disease Division of this hospital. Previous regimens consisted of isoniazid, streptomycin, and PAS. These drugs were being administered in various combinations. For this particular study PAS therapy was discontinued and the calcium benzoyl salt substituted, 4 gm. with each meal. The plasma concentrations for benzoyl PAS, PAS, and meta-aminophenol were essentially the same as those found in the controls. Although these patients were on continuous treatment, there were no detectable quantities of benzoyl PAS, PAS, and meta-aminophenol in the fasting blood samples, indicating complete clearance between the evening meal and 8:00 on the following morning.

Two patients were placed on sodium PAS, 12 gm. per day. The quantities of PAS and meta-aminophenol in plasma and urine are summarized in table 4. Plasma PAS concentrations were highest directly after feeding, and excretion was rapid. In these 2 patients, as with the controls receiving sodium PAS, the quantity of meta-aminophenol excreted

was greatly in excess of that theoretically expected, a twenty-five to thirty-two fold increase.

* * *

The concentrations of benzoyl PAS obtained in the plasma, even on regimens of 12 gm. of the calcium salt per day are rarely in excess of 5 mg. per 100 ml. Other investigators have found that, with three doses of 5 gm. each, blood concentrations of 12 mg. per 100 ml. can be attained.[4] Single-dose experiments indicate that maximal blood concentrations are reached within two to four hours after oral administration. There is a correlation between the concentration of drug in the blood and its excretion. Both are maximal at approximately the same time.

Benzol PAS and PAS can be detected in urine within an hour after the drug has been taken (shortest time interval employed), although peak concentrations do not usually occur until the second hour. There are occasional exceptions to this relationship.

After a single dose of drug, the highest plasma concentration is followed by a gradual diminution in the concentratior of both benzoyl PAS and its derivatives, although some benzoyl PAS is present after seven hours. However, over a longer period (6:00 p.m. to 8:00 a.m., the blood is completely cleared.

The quantities of calcium benzoyl PAS used in this study as a single dose or in multiple doses were not sufficient to establish meta-aminophenol as a product of benzoyl PAS metabolism. However, twice the equivalent quantity of calcium benzoyl PAS as of sodium PAS provided a readily available source of PAS and in sufficient concentration to result in the enzymatic decarboxylation of PAS to meta-aminophenol. If the reactions involved in benzoyl PAS metabolism are dependent on the presence of critical quantities of certain components, as suggested by the present data, then the individual metabolites eventually synthesized will be controlled by (*1*) the quantity of calcium benzoyl PAS administered; (*2*) the rapidity of benzoyl PAS absorption from the intestinal tract; (*3*) the degree of accumulation of adequate concentrations of the critical components as affected by the rate of the enzymatic reactions involved; (4) the rapidity with which each component is cleared from the blood. As the metabolites of benzoyl PAS are synthesized, a certain fraction of each may be conjugated. The degree of conjugation will, in part, depend on the factors mentioned above and also on the availability of glycine, acetate, glucuronic acid, and sulfate.

In summary: On a daily regimen of a 12 gm. of calcium benzoyl PAS, the concentration of benzoyl PAS and its metabolites in the blood rarely exceed 5 mg. per 100 ml. of plasma. A large portion of benzoyl PAS was excreted unchanged with a somewhat smaller quantity hydrolyzed to and excreted as PAS. Little, if any, meta-aminophenol was formed. If PAS in plasma equals or exceeds a certain critical concentration, meta-aminophenol is synthesized. Benzoyl PAS, PAS, and meta-aminophenol were entirely cleared from the blood during the overnight period in human subjects.

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October 11, 1956

[4] Schonholzer, G., *et al.*: On the Bacteriology and Pharmacology of Benzoyl-Para-Aminosalicylic Acid: A Report from the Research Institute. Dr. A. Wander, Berne, Switzerland. Private Communication.

[5] The writers wish to thank Dr. Samuel Phillips, Chief, Pulmonary Disease Division, for his cooperation.

A RAPID MICROCULTURE METHOD FOR THE ISOLATION OF TUBERCLE BACILLI[1]

For detecting tubercle bacilli in contaminated material, it would be desirable to have a simple and rapid microculture method which could form part of the routine laboratory cultural methods. Any micro-method which can give a positive result in seven days inevitably depends upon the microscopic finding of clusters of tubercle bacilli. The macroscopic appearance of colonies of tubercle bacilli on solid culture media, which is important for final diagnosis, usually requires another two weeks or more. However, an early report of positive findings by microculture can be of great help to the clinician, and in actual practice the reliability of microcultures is very good. For example, in 1,017 liquid cultures for the diagnostic isolation of tubercle bacilli, Armstrong[2] found 274 positives, none of which was atypical when subcultured on solid medium.

The method outlined below is simplified from that of Hedgecock and Faucher.[3] In their procedure, sediment from sputum treated by the sodium hydroxide method is washed in Trypticase® broth (B. B. L.) and then dried on a sterile slide, care being taken to avoid contamination. The slide is then placed, inoculated side down, on the surface of Tarshis blood agar medium.[4] After seven days the slide is removed, washed, and stained.

The present writers have found it possible to simplify the method, so that it is not necessary to wash the sediment or to dry it on a slide.

Method: The slides to be used are dipped in dilute serum (5 per cent normal human serum in distilled water), allowed to drain and dry, and then are autoclaved in a Petri dish or other container. By using a slide thinly coated with serum, the microcolonies adhere better than they do to an untreated slide. The neutralized sediment from sputum treated by sodium hydroxide or sodium triphosphate is inoculated in the usual way onto Tarshis blood agar medium (preferably leaving the tube with the surface of the medium horizontal) and incubated. A few hours later, or on the next day, using sterile forceps, a sterile slide is placed on the inoculated surface of the medium and pressed firmly to make good contact with the medium, and the sample is returned to the incubator. After about seven days the slide is removed, and placed in 1 per cent phenol for one hour, which washes and sterilizes it. After drying, the slide is fixed by heat, stained, and examined under the low-power objective for clusters of tubercle bacilli. As mentioned by Hedgecock and Faucher, the forceps should be rinsed in alcohol and flamed between each use.

In the writers' experience the method as outlined has given better results than that described by Hedgecock and Faucher, probably because it avoids the necessity of drying the specimen, a treatment which may impair the viability of some of the bacilli. This simple method, by avoiding the drying of the treated sediment on slides, removes one of the steps which could involve hazard to the technician and saves time.

It will be noted that, except for placing a sterile slide in the culture tube and removing it for staining a week later, no extra work is involved more than in the routine concentration and culture method. After removing the slide, the culture tube or bottle is returned to the incubator, so that macroscopic colonies can develop and be observed later.

On medium rich in blood, such as Tarshis or Bordet Gengou medium, tubercle bacilli

[1] This study forms part of a research project supported by a Canadian Federal Health Grant.

[2] Armstrong, A. R.: Canad. J. Med. Technol., 1950, *12*, 60.

[3] Hedgecock, L. W., and Faucher, I. O.: Am. Rev. Tuberc., 1956, *73*, 576.

[4] Tarshis, M. S., *et al.*: J. Bact., 1953, *66*, 448.

seem to grow just as well between the medium and the glass as they do on the surface of the medium. Hence, only medium with a high content of blood is likely to be satisfactory for this method.

Any size of culture tube or bottle, with a slide of a size to fit into it, may be used. A convenient container is the screw-cap bottle referred to as "2 ounce square," about 1⅜ by 1⅜ by 3½ inches; the present writers have had slides cut down to fit into the bottles. A modified staining rack is needed for these smaller slides.

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February 15, 1957

Books

SUBPHRENIC ABSCESS. By H. R. S. Harley. Pp. 216, Charles C Thomas, Springfield, Illinois, 1956. Price, $7.00.

This monograph is a carefully organized treatise which is concerned with a relatively neglected subject, subphrenic abscess. The presentation is based on the author's study of 188 patients. His statements are drawn from his own observations in this series and are analyzed in regard to findings that have appeared previously in medical literature.

After a brief section on the historic aspects of subphrenic abscess, the author presents a description of the anatomy of the subphrenic region. This chapter is written in a lucid manner and is particularly valuable. The subsequent chapters are devoted to the etiology of subphrenic infection, the routes and mode of spread of infection, and the cause, mechanism, and incidence of intrathoracic extension. The text is supplemented by tables and line drawings.

Later chapters are concerned with clinical findings, diagnostic aspects, and treatment of subphrenic abscess. The roentgenographic changes are discussed in detail. The importance of the clinical findings in diagnosis and localization of the abscess is stressed. Emphasis is placed on diagnostic significance of pain which is a common and important symptom.

The surgical approach that is described is based on sound fundamental principles. The danger of diagnostic aspiration outside of the operating room is indicated. The author advocates extraserous drainage of abscesses and presents the proper approaches in diagrammatic form.

An adequate bibliography is included. There are 41 figures and 51 tables which support the text.

There are two areas in which the value of this dissertation might be enhanced. Rather meager bacteriologic data are provided and consideration of antimicrobials is limited to penicillin. Much of the clinical material apparently antedated the use of the more recent antimicrobial agents. The placement of the reproductions of the roentgenograms in the back of the book makes reference to them awkward.

The book is an excellent reference for subphrenic abscess and should be of value for both students and clinicians.

JOHN M. BEAL
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IMMUNOLOGY AND SEROLOGY. By Philip L. Carpenter. Pp. 351, W. B. Saunders Company, Philadelphia, 1956. Price, $6.50.

This compact volume is a good text for the shelf of the student who is beginning the study of immunology and serology. It is clearly written and the material is presented in a well-organized fashion, with many subheadings to divide each chapter into smaller units. The historic approach to many of the subjects adds interest and broadens the reader's perspective of the topics under discussion. Many excellent tables and charts illustrate the text.

The chief criticism of this book is the absence of discussion on some of the important recent developments in the field of immunology, such as the properdin system, immunologic tolerance, agammaglobulinemia and its immunologic significance, and gel diffusion

techniques. In addition, there are insufficient recent references for the student who wishes to probe some subjects more deeply.

CHARLOTTE MARKER ZITRIN
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DIE LUNGENTUBERKULOSE. Diagnose und Therapie. Third edition. By Paul-George Schmidt. Pp. 384, Georg Thieme Verlag, Stuttgart, 1956.

This extensive monograph gives a detailed review of the diagnosis and treatment of pulmonary tuberculosis in Germany together with a thorough discussion of the social problems involved. The book begins with a well-written introduction to the history of the disease, presenting an interesting picture of the numerous theories of its origin and the even more numerous attempts to treat it. A chapter on the morphology of the tubercle bacillus makes the reader acquainted not only with the most recent achievements in research, but also with older, widely discussed theories (Calmette's ultra-virus, et cetera).

The chapter on staining methods is limited only to the most widespread methods after Ziehl-Neelsen and the fluorescence microscopy. The same applies to the chapter on the culture of the bacillus, in which a more detailed description of the most important media would be of avail. A large number of good pictures illustrates an extensive discussion of both the morphology and the histology of the tuberculous lesion, followed by a description of the immunologic features, in which mainly German authors such as Petruschky, Moro, Pirquet, Roessle, Ranke, and Pagel are considered.

A chapter on BCG vaccination leads to the clinical section, which begins with a treatise on diagnosis and differential diagnosis. The comparatively rare occurrence of sarcoidosis and pulmonary fungal diseases in Germany is reflected by the fact that the former is only briefly mentioned and the latter is not mentioned at all. The author considers sarcoidosis to be an atypical form of pulmonary tuberculosis. The course of both pulmonary and complicating extrapulmonary forms of the disease are thoroughly discussed, based upon clinical and statistical data taken from his own experience and the European literature. Consequently, all available methods of treatment are discussed. Dr. Schmidt emphasizes the importance of rest but recommends that the patient have exercise as soon as possible, depending upon the individual case.

Dr. Schmidt shares the American experiences in treatment with antimicrobials and chemotherapy, having observed frequent relapses in single-drug therapy. In his opinion, single-drug therapy should be replaced by multiple-drug therapy of the alternating type; for instance: one month, streptomycin and isoniazid; one month, isoniazid and PAS; one month, streptomycin and PAS; et cetera. Streptomycin is used in a dose of 1 gm. per day, isoniazid, 8 to 10 mg. per kg. per day. PAS is frequently administered as intravenous infusion up to 40 gm. per day.

The author's vast experience is reflected by an extensive discussion of the surgical approach. In collapse therapy he limits both pneumothorax and pneumoperitoneum to a few, well-selected indications, i.e., a small elastic cavity after primary infiltration, and small nodular dissemination in the upper lobes if antimicrobial treatment has proved to be of no value. He claims to have had good and dependable results with thoracoplasty (two-stage procedure with removal of the first to the seventh ribs), which can be combined with either a Monaldi or Maurer type of cavity drainage. He advocates resection in cases of tuberculoma, perforation of a cavity, 'destroyed lung,' and rest-cavities (thick-walled cavities), particularly those of the lower lobe. Hemorrhage, bronchial fistula, emphysema, pulmonary fistula and empyema are mentioned as complications.

An excellent presentation of the available statistical data on mortality and morbidity concludes the book. A comparison with American statistics can be made only with some difficulty because of the difference in characterizing the different stages of the disease, but the over-all impression is that the morbidity does not show any significant change, while the mortality per 10,000 living persons has reached a previously unobserved low point of 1.78.

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BOOKS RECEIVED

LA MUTAZIONALITA DEL MYCOBACTERIUM TUBERCULOSIS. By G. Petragnani. Pp. 75, Federazione Italiana Contro la Tubercolosi, Rome, 1956.

CHRONIC BRONCHITIS: An NAPT Symposium. Pp. 44, Waterlow and Sons, Ltd., London, 1957. Price, $1.50.

PRÉVENTION ET TRAITEMENT SPÉCIFIQUES DE LA TUBERCULOSE. By Léopold Nègre. Pp. 242, Masson et Cie., Paris, 1956. Price, 1.800 fr.

DICTIONARY OF MICROBIOLOGY. By Morris B. Jacobs, Maurice J. Gerstein, and William G. Walter. Pp. 276, D. Van Nostrand Company, Inc., Princeton, 1957. Price, $6.75.

EXPECTANT MOTHERHOOD. By Nicholson J. Eastman. Pp. 198, Little, Brown and Company, Boston, 1957. Price, $1.75.

CLINICAL CARDIOPULMONARY PHYSIOLOGY. Edited by Burgess L. Gordon. Pp. 759, Grune and Stratton, Inc., New York, 1957. Price, $15.75.

LIVER, BILIARY TRACT AND PANCREAS. Vol. 3, Part III: Digestive System. Prepared by Frank H. Netter, edited by Ernst Oppenheimer. Pp. 165, CIBA, Summit, New Jersey, 1957. Price, $10.50.

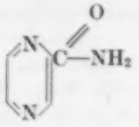
American Trudeau Society

Medical Section of the National Tuberculosis Association

A Statement on Pyrazinamide

By the Committee on Therapy

Pyrazinamide, pyrazinoic acid amide, is similar in structure to nicotinamide. It was synthesized by Kushner and associates (1) after it had been demonstrated by McKenzie and co-workers (2) that nicotinic acid amide had a suppressive action on the course of experimental tuberculosis induced in white mice.



Studies using animals (3) revealed that pyrazinamide had a very low toxicity, even when administered in rather large doses. However, a dog receiving 1.0 gm. of pyrazinamide per kg. of body weight showed abnormal liver function tests and, after fifty-eight days of treatment, developed massive hepatic necrosis. Clinical studies have revealed that the drug is much more toxic for humans than it is for animals, and it is this toxicity which will probably limit its clinical usefulness.

In vitro action of pyrazinamide: The early reports on the *in vitro* effects of pyrazinamide were inconsistent, and a marked variation of activity was apparent from one culture medium to another. For example, Tarshis and Weed (4) could demonstrate no inhibitory effect of 100 mg. of pyrazinamide per ml. in solid American Trudeau Society (ATS) medium, whereas Dessau and associates (5) found that 100 mg. per ml. was inhibitory in Dubos liquid Tween®-albumin medium. McDermott and Tompsett (6) shed some light on this problem by showing that the pH of the medium affects the activity of pyrazinamide and that an acidic environment (pH 5.0–5.5) is necessary for the drug's action.

In vitro studies of bacterial resistance to pyrazinamide (7) reveal that marked resistance to the drug can be produced in as few as three transfers. Solotorovsky and Ironson (8) have shown that *in vitro* pyrazinamide will retard the appearance of resistance to isoniazid. These experimental findings are substantiated in the clinical trials.

Animal experiments: Preliminary studies (9, 10) with mice and with guinea pigs established that the tuberculostatic activity of pyrazinamide is somewhat more effective than that of PAS but is definitely inferior to that of streptomycin. Subsequent studies by Steenken and Wolinsky (11) indicated that pyrazinamide alone has a very weak antituberculous action against experimentally induced tuberculosis in guinea pigs. However, their experiments ran for a prolonged period, and the possibility exists that drug-resistant organisms developed, escaped from the action of pyrazinamide, and caused extension of the disease in treated animals.

The most interesting and thought-provoking experiments, however, have been those of Tompsett and co-workers (12, 13) who employed the white mouse as an experimental animal and a bacterial enumeration of the spleen as their statistical tool to show that pyrazinamide possesses the potential not just to arrest, but to eradicate, infection. These experiments only lend weight to other evidence that pyrazinamide has useful potentialities in clinical medicine, but they do not imply that the drug can have an eradicative

action on human tuberculosis. In the mouse experiments described, the dose of drug administered was extremely large, and the spleen was selected as an indicator organ for the study although isoniazid alone or in combination with PAS and/or streptomycin could cause tubercle bacilli to vanish from the lung. McDermott and associates (14) have also reported that, although isoniazid-pyrazinamide treatment appeared to eradicate bacilli from the mouse spleen, approximately one-third of the animals had infected spleens ninety days after therapy was stopped. Hence, one must consider that the infection was latent, not eradicated.

Clinical studies: The first clinical trial of pyrazinamide was conducted by Yeager and associates (15). They finally employed a daily dose of 2.8 gm., although one patient was given 8.4 gm. per day for a thirty-three day period. Forty-three patients were treated and the following information was obtained. The drug appeared to exert a moderate antituberculosis effect; 10 of 33 patients had sputum conversion by direct smear, and most of the patients experienced lessening of cough and sputum. It was apparent, however, that the drug's effect was not sustained and that resistance to pyrazinamide developed rapidly.

Ten patients developed joint pains and this is probably a significant observation in the light of recent reports (16, 17) that pyrazinamide therapy results in elevated blood uric acid and that, in some patients, frank clinical gout can develop. The drug's major toxic effect was also discovered in this first trial. Two of the 43 patients became jaundiced and this hepatotoxic effect is the one that has most discouraged the general use of pyrazinamide in clinical medicine. The hepatic toxicity is sometimes progressive and irreversible and deaths have resulted from the use of pyrazinamide. Liver function tests do not always give adequate warning of impending liver damage. Subsequent clinical trials have only served to confirm the observations of the first trial. These clinical reports are summarized in the accompanying table.

Author	Reference	Number of Cases	Jaundice	Other Toxic Effects
Yeager *et al.*	15	Pyrazinamide alone: 43	2	Joint pains: 11
McDermott *et al.*	18	Pyrazinamide and isoniazid: 55	5	
Campagna *et al.*	19	Pyrazinamide and isoniazid: 21	3	Joint pains: 10
Phillips *et al*	23	Pyrazinamide alone: 11; pyrazinamide and other drugs: 18	1	
Schwartz and Moyer	24	181	2	
Krasner	25	Pyrazinamide and PAS: 10	0	
Salkin and Schwartz	26	23	0	
Potter and Chang	27	60	8	Hemoptysis: 8
Muschenheim	21	Low dosage pyrazinamide: 61	2	
Phillips and Horton	20	Pyrazinamide and isoniazid: 38	2	Bromsulphalein increased: 3
Pfuetze and Pyle	28	Pyrazinamide and viomycin: 34	0	
Desautels	29	Pyrazinamide and isoniazid: 42	1	Bromsulphalein increased: 4
Schwartz	30	472	7	Bromsulphalein increased: 36

The use of pyrazinamide combined with streptomycin or with isoniazid may have particular value. Apparently, it enhances the action of, and delays resistance to, the drug with which it is combined. Of special interest is the combination of isoniazid and pyrazinamide as demonstrated by the studies of McDermott and associates (18) and of Campagna and co-workers (19). In both McDermott's and Campagna's series sputum conversion was 90 per cent. However, a proper evaluation of this combination would re-

quire a randomized group of patients treated with pyrazinamide and isoniazid and compared with a similar group of patients treated with PAS and isoniazid. Such a series is reported by Phillips and Horton (20), and at eight months there was no significant statistical difference between the two series. It is of interest that none of the pyrazinamide-isoniazid-treated patients developed isoniazid-resistant organisms.

The beneficial effects derived from pyrazinamide plus isoniazid depend, however, upon an adequate dose of pyrazinamide. Muschenheim and co-workers (21) report that halving the dose of pyrazinamide resulted in inferior therapeutic activity and did not eliminate the drug's toxicity.

In conclusion, pyrazinamide used alone has only a limited effect. Its serious hepatotoxic action tends to outweigh its therapeutic value and limits the use of this drug to patients who have ceased to respond to other antituberculosis drugs, who are undergoing surgery without the benefit of other drug coverage, or who cannot tolerate other antituberculosis drugs because of hypersensitivity reactions. It cannot be recommended for initial treatment or for long-term therapy.

Patients who receive this drug should have preliminary liver function studies and a blood uric acid determination. Serum glutamic oxalacetic transaminase determinations have been reported (22) to be a highly sensitive test of early liver damage in pyrazinamide-treated patients. Liver function tests should be repeated at frequent intervals. However, such studies do not always give adequate warning of impending liver damage, and daily clinical observations for such manifestations as anorexia, nausea, malaise, jaundice, fever, liver enlargement, and liver tenderness should be made. The adult dose of pyrazinamide is 750 mg. four times daily (40 to 50 mg. per kg. of body weight). Doses lower than this appear to compromise the drug's therapeutic effect without abolishing its toxic effect.

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REFERENCES

(1) Kushner, S., Dalalian, H., Sanjuro, J. L., Back, F. L., Safir, S. R., Smith, V. K., and Williams, J. H.: Experimental chemotherapy of tuberculosis: II. Synthesis of pyrazinamide and related compounds, presented before the Medicinal Chemistry Section, American Chemical Society, Milwaukee, Wisconsin, April 1, 1952.

(2) McKenzie, D., Malone, L., Kushner, S., Oleson, T. J., and Subbarow, Y.: The effect of nicotinic acid amide on experimental tuberculosis in white mice, J. Lab. & Clin. Med., 1949, *33*, 1249.

(3) Robinson, H. J., Siegal, H., and Pietrowski, T. J.: Toxicity of pyrazinamide, Am. Rev. Tuberc., 1954, *70*, 423.

(4) Tarshis, M. S., and Weed, W. A. J.: Lack of significant *in vitro* sensitivity of *Mycobacterium tuberculosis* to pyrazinamide on three different solid media, Am. Rev. Tuberc., 1953, *67*, 391.

(5) Dessau, F. I., Yeager, R. L., Burger, F. J., and Williams, J. H.: Pyrazinamide in experimental tuberculosis in the guinea pig, Am. Rev. Tuberc., 1952, *65*, 519.

(6) McDermott, W., and Tompsett, R.: Activation of pyrazinamide and nicotinamide in acidic environments *in vitro*, Am. Rev. Tuberc., 1954, *70*, 748.

(7) Solotorovsky, M., Gregory, F. J., Ironson, E. J., Bugie, E. J., O'Neill, R. C., and Pfister, R., III: Pyrazinoic acid amide: An agent active against experimental murine tuberculosis, Proc. Soc. Exper. Biol. & Med., 1952, *79*, 563.

(8) Solotorovsky, M., and Ironson, E. J.: Retarding effect of pyrazinamide on emergence of resistance of the tubercle bacillus to isoniazid, Tr. Thirteenth Conference

on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1953, p. 172.

(9) Malone, L., Schurr, A., Lindh, H., McKenzie, D., Kiser, T. S., and Williams, J. H.: The effect of pyrazinamide on experimental tuberculosis in mice, Am. Rev. Tuberc., 1952, *65*, 511.

(10) Dessau, F. I., Yeager, R. L., Burger, F. J., and Williams, J. H.: Pyrazinamide in experimental tuberculosis of guinea pig, Am. Rev. Tuberc., 1952, *65*, 519.

(11) Steenken, W., Jr., and Wolinsky, E.: The antituberculous activity of pyrazinamide *in vitro* and in the guinea pig, Am. Rev. Tuberc., 1954, *70*, 367.

(12) Tompsett, R., McCune, R. M., Jr., Ormond, L., Deuschle, K., and Muschenheim, C.: Influence of PZA-INH on *M. Tuberculosis* in animals and man, Trans., A. Am. Physicians, 1954, *67*, 224.

(13) Wasz-Höckert, O., McCune, R. M., Jr., Lee, S. H., McDermott, W., and Tompsett, R.: Resistance of tubercle bacilli to pyrazinamide *in vivo*, Am. Rev. Tuberc., 1956, *74*, 572.

(14) McDermott, W., McCune, R. M., Jr., and Tompsett, R.: Dynamics of antituberculous chemotherapy, Am. Rev. Tuberc., 1956, *74* (Supplement, p. 100).

(15) Yeager, R. L., Munroe, W. G. C., Dessau, F. I.: Pyrazinamide in the treatment of pulmonary tuberculosis, Am. Rev. Tuberc., 1952, *65*, 523.

(16) Quarterly Progress Report of the Veterans Administration-Armed Forces Study on the Chemotherapy of Tuberculosis, July, 1956, Albany, New York, p. 7.

(17) Cullen, J. H., Early, L. J. A., and Fiore, J. M.: The occurrence of hyperuricemia during pyrazinamide-isoniazid therapy, Am. Rev. Tuberc., 1956, *74*, 289.

(18) McDermott, W., Ormond, L., Muschenheim, C., Deuschle, K., McCune, R. M., Jr., and Tompsett, R.: Pyrazinamide-isoniazid in tuberculosis, Am. Rev. Tuberc., 1954, *69*, 319.

(19) Campagna, M., Calix, A. A., and Hauser, G.: Observations on the combined use of pyrazinamide and isoniazid in the treatment of pulmonary tuberculosis, Am. Rev. Tuberc., 1954, *69*, 334.

(20) Phillips, S., and Horton, G. E.: Pyrazinamide-isoniazid, Am. Rev. Tuberc., 1956, *73*, 704.

(21) Muschenheim, C., Organick, A., McCune, R. M., Jr., Batten, J., Deuschle, K., Tompsett, R., and McDermott, W.: Pyrazinamide-isoniazid in tuberculosis, Am. Rev. Tuberc., 1955, *72*, 851.

(22) Transactions of the Sixteenth Conference on the Chemotherapy of Tuberculosis held by the Veterans Administration-Army-Navy, February, 1956.

(23) Phillips, S., Larkin, J. C., Jr., Litzenberger, W. L., Horton, G. E., and Haimsohn, J. S.: Pyrazinamide in pulmonary tuberculosis, Am. Rev. Tuberc., 1954, *69*, 443.

(24) Schwartz, W. S., and Moyer, R. E.: The chemotherapy of pulmonary tuberculosis with pyrazinamide, used alone and in combination with streptomycin, PAS, or isoniazid, Am. Rev. Tuberc., 1954, *70*, 413.

(25) Krasner, L.: Pyrazinamide in Ten Cases of Chronic Pulmonary Tuberculosis, Tr. Twelfth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1953, p. 341.

(26) Salkin, D., and Schwartz, J. A.: Pyrazinamide Studies, Tr. Twelfth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1953, p. 343.

(27) Potter, B. P., and Chang, S. F.: Experiences with pyrazinamide, Dis. of Chest, 1955, *27*, 44.

(28) Pfuetze, K., and Pyle, M.: Personal communication.

(29) Desautels, G.: Quarterly Progress Report of the Veterans Administration-Armed Forces Study on the Chemotherapy of Tuberculosis, July, 1956, p. 31.

(30) Schwartz, O.: Report to the Quarterly Progress Report of the Veterans Administration-Armed Forces Study on the Chemotherapy of Tuberculosis, July, 1956, p. 58.

A Statement on Cycloserine

By the Committee on Therapy

Cycloserine is an antibiotic produced by *Streptomyces orchidaceus*. It inhibits the growth of human tubercle bacilli including those resistant to streptomycin and isoniazid in concentrations of 5 to 20 micrograms per milliliter (1) and bovine and avian strains in somewhat higher concentrations (2, 3).

Various workers (3–5) demonstrated that cycloserine has no beneficial effect on either the survival time or the extent of gross disease in experimentally infected mice or guinea pigs. Cycloserine produces a definite but limited beneficial effect against experimental tuberculosis in rabbits (6) and in monkeys (7). This species' difference is more conspicuous than in most other drugs. It may be related to metabolism of the drug and its excretion (7) or to variations in host factors (8).

Cycloserine has been administered orally to adults in doses of 0.5 to 1.5 gm. per day in divided doses. It has certain definite toxic reactions (9). The major manifestations of toxicity are central nervous symptoms either excitatory, including major convulsive disorders, or depressant. Frank psychoses have been produced. When doses larger than 25 mg. per kg. are given, serious toxic reactions can be expected in nearly all cases (10). The major central nervous reactions have appeared early, occasionally during the first week, and usually during the first month. In some instances, however, this reaction may occur later. Frequency and severity of reactions seem related to peak blood levels. Other toxic reactions have been relatively unimportant. They have included skin eruptions and fever.

Cycloserine used alone has been reported to be an active antibiotic in the treatment of previously untreated cases of active pulmonary tuberculosis in the dosage above described (11, 12). It has been reported to be of value in re-treatment of patients apparently resistant to other compounds. Certain studies have suggested, however, that its action is less potent than either streptomycin or isoniazid.

In therapy with cycloserine alone it has been shown that resistant bacilli may develop *in vitro* and in treated patients in a relatively short period of time (13, 14). It has been reported that such emergence of resistance is associated with clinical deterioration. It has not yet been determined whether this emergence of resistance may be delayed by concurrent administration of other agents.

At this time, although cycloserine is available commercially, knowledge concerning its clinical use is far from complete. At present the only established indication for its use is in those patients who fail to respond despite the use of other antituberculosis drugs. Current studies of cycloserine in lower dosage than hitherto employed and in combination with isoniazid will determine whether it will have a wider indication. Its use should be restricted to hospitalized patients. It should be immediately discontinued with the appearance of central nervous system disorders. Particular precautions should be taken in renal disfunction.

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REFERENCES

(1) Harned, R. L., Hidy, P. H., and La Baw, E. K.: Cycloserine 1: A preliminary report, Antibiot. & Chemother., 1955, *5*, 204.

(2) Cummings, M. M., Patnode, R. A., and Hudgins, P. C.: Effects of cycloserine on *Mycobacterium tuberculosis in vitro*, Antibiot. & Chemother., 1955, *4*, 198.

(3) Steenken, W., Jr., and Wolinsky, E.: Cycloserine: Antituberculous activity *in vitro* and in the experimental animal, Am. Rev. Tubere., 1956, *73*, 539.

(4) Patnode, R. A., Hudgins, P. C., and Cummings, M. M.: Effect of cycloserine on experimental tuberculosis in guinea pigs, Am. Rev. Tubere., 1955, *72*, 117.

(5) Youmans, G. P.: The use of the mouse for the testing of chemotherapeutic agents against *Mycobacterium tuberculosis*, Ann. New York Acad. Sc., 1949, *72*, 662.

(6) Runyon, E. H., and discussion by Anderson, R. C., Worth, H. M., Welles, J. S., Harris, P. N., and Chen, K. K.: Pharmacology and toxicology of cycloserine, Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 350.

(7) Schmidt, L. H.: Studies on the therapeutic properties of cycloserine, Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 353.

(8) Mulinos, M. G.: Cycloserine: An Antibiotic Paradox, Antibiotics Annual, 1955–1956, Medical Encyclopedia, Inc., New York, New York, p. 133.

(9) Murray, F. D.: A pilot study of cycloserine toxicity, Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 333, and other articles in same issue.

(10) Barclay, W. R., Pyle, M., Poirier, P., and Pfuetze, K.: High dosages of cycloserine plus phenobarbital and Dilantin for the treatment of pulmonary tuberculosis, presented at Sixteenth Veterans Administration-Armed Forces Conference on the Chemotherapy of Tuberculosis, February 11–14, 1957.

(11) Epstein, I. G., Nair, K. G. S., and Boyd, L. J.: Cycloserine, a new antibiotic in the treatment of human pulmonary tuberculosis: A preliminary report, Antibiot. Med., 1955, *1*, 80.

(12) Epstein, I. G., Nair, K. G. S., Bachman, M., Baldwin, R., and Mulinos, M. G.: Treatment of human tuberculosis with cycloserine: Combined therapy, Tr. Fifteeth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 365.

(13) Moyer, R. E., and Schwartz, W. S.: Development of bacterial resistance to cycloserine and its correlation with clinical and x-ray changes, Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 380.

(14) Cummings, M. M.: Cycloserine resistance data, Tr. Fifteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1956, p. 377.

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The following date and location of the next annual meeting of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

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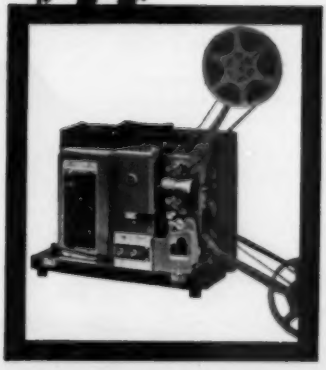

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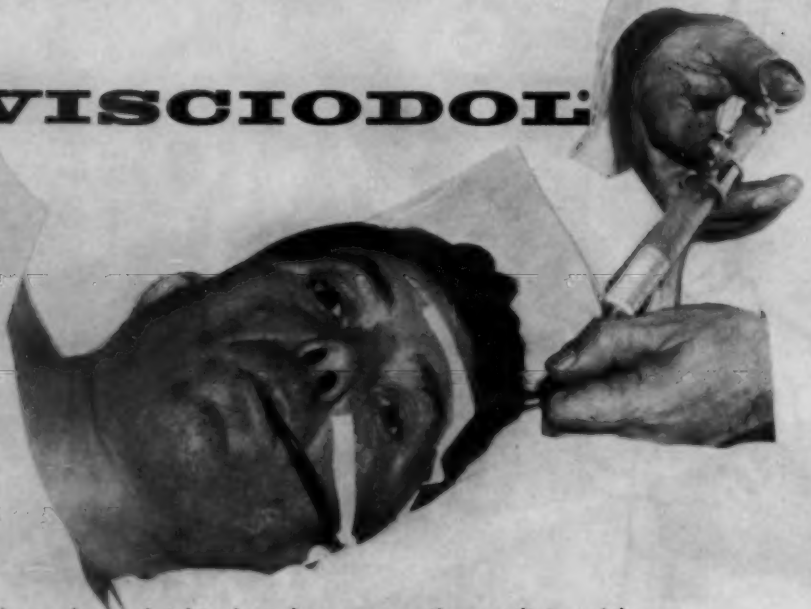

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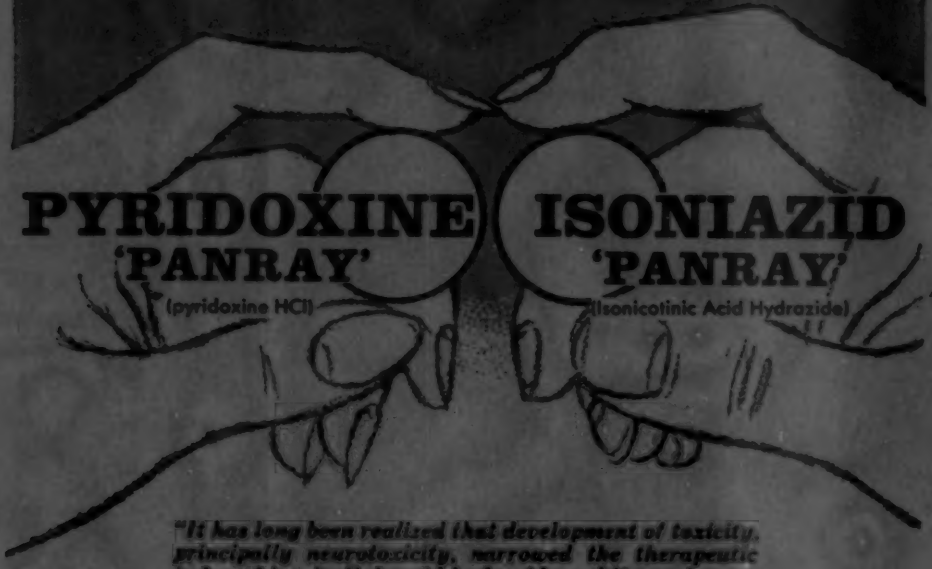

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